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AN EXPERIMENTAL BRAIN MISSILE WOUND:
ASCERTAINING PATHOPHYSIOLOGY AND EVALUATING TREATMENTS TO LOWER MORTALITY AND MORBIDITY

ANNUAL REPORT

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Brain missile wounding (BMW) is accompanied with a concomitant increase in intracrania pressure (ICP). Both, BMW with its accompanying increase in ICP or artificially increased ICP, without wounding, caused significant epinephrine (EPI) depletions (47%-68%) in the nucleus tractus solitarius (NTS), area A1C1, locus coeruleus, raphe nuclei and posterior hypothalamus. EPI was also significantly decreased in the anterior hypothalamus but only in wounded cats. BMW, but not artificially increased ICP, without wounding, also caused significant decreases of norepinephrine (NE), dopamine and homovanillic acid the NTS and area A1C1. We conclude that most of the brain stem and hypothalamic biogenic amine changes were probably caused by the cardiovascular effects associated with the stress of increased ICP alone. The results suggest selective monoamine decreases associated with increased ICP: the EPI system overall and the 5-HT system in the raphe nuclei. There remains the possibility that NE reductions in the NTS and area A1C1 found only in the wounded cats are the result of

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the very high instantaneous overpressures and resultant pressure waves in the cranial vault as a result of BMW.

The disruption of mechanical cerebral blood flow regulation occurs from brain missile wound effects per se, not from the associated increase in ICP.

SUMMARY

This Annual Report covers two areas: 1) the effect of brain missile wounding on hypothalamic and brainstem biogenic amines; 2) the effect of increased intracranial pressure (ICP) on mechanical cerebral blood flow (CBF) regulation.

1) We have ascertained the acute effects of brain missile wounding on brain stem and hypothalamic biogenic amines epinephrine (EPI), norepinephrine (NE), and 5 hydroxytryplophane (5-HT) and their metabolites DOPAC, homovanillic acid (HVA), 5 hydroxyindoleacetic acid (5-HIAA) in pentobarbital anesthetized cats. We have already shown the effects of elevated ICP alone on these brain stem and hypothalamic biogenic amines and metabolites. With both brain missile wounding and artificially-induced increases in ICP, significant EPI depletions (47%-68%) occurred in the nucleus tractus solitarius, area A1C1, locus coeruleus, raphe nuclei and posterior hypothalamus. EPI was also significantly decreased in the anterior hypothalamus but only in wounded cats. Both brain wounding and artificially induced increased in ICP also caused significant decreases of norepinephrine in the posterior hypothalamus. Serotonin (5-HT), 5-hydroxyindoleacetic acid, dopamine (DA) and homovanillic acid (HVA) were decreased in the raphe nuclei. Only brain wounding caused significant reductions of NE, DA, and HVA in the nucleus tractus solitarius and area A1C1. We conclude that most but not all of the selective brain stem and hypothalamic biogenic amine changes were caused by cardiovascular effects associated with the stress of increased ICP alone whether caused by wounding or by artificially increasing the cerebrospinal fluid pressure.

2) We previously ascertained that brain missile wounding severely affected mechanical CBF regulation. Because brain missile wounding also raises ICP we could not discern whether it was the wounding per se or the associated ICP which disturbed mechanical CBF regulation. In the present experiments on pentobarbital-anesthetized cats we show that ICP increases by themselves do not destroy mechanical CBF regulation. The loss of mechanical CBF regulation following brain wounding results from the wound process itself, not the associated ICP increase.

This effect of either an ICP increase or brain wounding on brain stem hypothalamic biogenic amines contrasts to mechanical CBF regulation which is destroyed by brain wounding but not by an elevated ICP alone.

FOREWORD

In conducting research using animals, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23 Revised 1985).

Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

The experiments presented in this report were performed by Joseph Soblosky, Ph.D., Lynn Rogers, M.D., Jeffrey Adams, M.D., (biogenic amines); Dan Torbati, Ph.D., June Davidson, B.S. (ICP mechanical CBF regulation).

This manuscript was typed by Mrs. Elizabeth P. Hulbert.

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EFFECTS OF BRAIN MISSILE WOUNDING
ON BRAIN BIOGENIC AMINES

INTRODUCTION

Severe head trauma and brain injury may cause axial brain stem displacement and are often followed by an increase in intracranial pressure (ICP), systemic arterial hypertension, respiratory changes and a generalized sympathetic response. The increase in mean arterial blood pressure (MABP) and sympathetic responses as a result of increased ICP has been termed the Cushing pressor response (12,13).

Biogenic amines (norepinephrine, NE; epinephrine, EPI; dopamine, DA; serotonin, 5-HT) are thought to be important neurotransmitters in the hypothalamus and in brain stem cardiovascular and respiratory control centers[20,44]. Drug- or blood volume-induced alterations in blood pressure have been correlated with changes in monoamine levels in the nucleus tractus solitarius (NTS) (3,29), rostral and caudal ventrolateral medulla (5,27,48), locus coeruleus (LC) (4), hypothalamus (45,60) and dorsal raphe nucleus (15,17).

Studies examining the effects of cortical lesions or ischemia on biogenic amines have found alterations which can be correlated with subsequent pathophysiology and/or behavioral dysfunctions (1,8,33,35,41,43,49,64,67). Most CNS trauma studies on the effects on monoamine neurotransmitters have utilized the spinal cord-injury model (6,31,37,39,57,69). A few studies have been performed determining the effects of brain trauma (16,25,28) or increased ICP (40) on brain biogenic amines, but only gross brain areas were sampled in these studies.

We utilize a brain missile wound (BMW) model wherein the feline brain is injured by a small pellet mimicking a low energy brain wound in the human (9). In our model of missile wounding to the brain there are apparent "brain stem effects" even though the cerebral hemisphere is the wound site i.e. the "brain stem effects" are an indirect effect of wounding. " Brain stem effects" i.e. disturbances in cardiovascular and respiratory functions are well noted following brain wounding and in our model dramatic increases in ICP and MABP occur (9). An immediate Cushing response (transient elevations in MABP, slight bradycardia and respiratory depression) is a prominent feature of the systemic response to BMW. At higher missile energies the respiratory depression may be so severe that fatal apnea occurs (9). Virtually instantaneous (microseconds) intracranial overpressures also occur following brain wounding which are associated with the propagation of pressure waves within the skull which may affect the brain at a distance from the wound itself. We have not measured these virtually instantaneous overpressures in our model.

Because brain biogenic amines have been seen to be perturbed following CNS injury and after blood pressure changes, we thought it important to determine whether biogenic amine changes occurred in the hypothalamus and brain stem following BMW which is invariably associated with increases in ICP and MABP.

In order to determine if the cardiovascular and respiratory
effects of missile wounds to the brain are solely attributable to
the accompanying increase in ICP, it was necessary to first
conduct experiments examining the effects of increased ICP alone,
without wounding. We have previously reported our results
evaluating biogenic amine changes in the brain stem
cardiovascular centers and the hypothalamus consequent to
increases in ICP, without wounding. We increased the ICP by
infusions of mock CSF into the cisterna magna in such manner as
to mimic as closely as possible the ICP response seen after BMW.
In those experiments we found dramatic decreases in epinephrine
(EPI) levels in every brain stem area examined as well as the
posterior hypothalamus. We also found decreases in
norepinephrine (NE) in the posterior hypothalamus in addition to
decreases in serotonin (5-HT), 5-hydroxyindoleacetic acid (5-
HIAA), dopamine (DA) and homovanillic acid (HVA) in the raphe
nuclei (see 1989 yearly report).

The present report evaluates the effects of missile wounds
to the brain on biogenic amines in the same brain stem areas and
the hypothalamus. The comparison of the present results with
those previously reported for increases in ICP, without wounding,
will aid in determining if the brain stem and hypothalamic
biogenic amine decreases are solely attributable to increases in
ICP alone, or if there are additional effects which are
attributable to other factors related to missile wounding.

MATERIALS and METHODS

A. CHEMICALS and REAGENTS

Norepinephrine bitartrate (NE), epinephrine bitartrate
(EPI), dopamine HCL (DA), 5-hydroxytryptamine creatinine sulfate
(5-HT, serotonin), 3,4-dihydroxyphenylacetic acid (DOPAC), 4-
hydroxy-3-methoxyphenylacetic acid (HVA), 5-hydroxyindoleacetic
acid (5-HIAA), normetanephrine HCl (NM), 3,4-dihydroxybenzylamine
hydrobromide (DHBA), monochloroacetic acid (MCA), ethylene-
diaminetetracetic acid (EDTA), sodium hydroxide (NaOH), dibasic
sodium phosphate (Na_2HPO_4), monobasic potassium phosphate
(KH_2PO_4), and Tris base were purchased from Sigma Chemical Comp.,
St. Louis, MO. Sodium octyl sulfate (SOS) was purchased from
Eastman Kodak Co., Rochester, NY. Perchloric acid ($HClO_4$) and
acetonitrile (CH_3CN) were obtained from J.T. Baker, Phillipsburg,
NJ. and hydrochloric acid (HCL) from Mallinckrodt, St. Louis, MO.
Acid-washed alumina oxide (AAO) was purchased from BAS, W.
Lafayette, IN. All water was either HPLC grade (J.T. Baker) or
double distilled and deionized prior to use. Reference standard
solutions of NE, EPI, DA, 5-HT, DOPAC, HVA, 5-HIAA, NM, and DHBA
were made using .05 M $HClO_4$ containing .1 mM EDTA and stored at
-70°C. Working standard solutions were prepared by serial
dilutions using the same diluent and stored at 4°C.

B. GENERAL SURGICAL PROCEDURES

Unselected, unfasted mongrel cats (3-5 kg) of either sex were used. Animals were first anesthesized with pentobarbital (40 mg/kg, i.p.). Adequacy of anesthesia was evaluated by cessation of limb withdrawal from thumb and index finger pinch between the toes and abolition of corneal reflex elicited by the touch of a paper wisp to the cornea. Arterial and venous cannulae (PE 90) were implanted in the right rear leg after treatment of the incision area with topical anesthetic (2% lidocaine). An endotracheal tube smeared with topical anesthetic (2% xylocaine jelly) was inserted after application of local anesthetic (0.5 ml 2% xylocaine) to the epiglottis. The cat was mounted in the stereotaxic frame (Kopf Inst., Tujunga, CA) and a MABP transducer (Narco, Houston, TX) was attached to the arterial cannula for physiograph recording (Narco). Expired CO_2 was measured by an end tidal CO_2/respiratory rate monitor (Instrumentation Lab., Watertown, MA) and also recorded on the physiograph. The depth of anesthesia was rechecked frequently during the experimental period and prior to sacrifice using the above criteria as well as the MABP and the respiratory rate. If required, the cats were given supplemental anesthesia (pentobarbital, 6.5 mg, i.v.). The head was partially shaved and a 5 cm scalp incision made. A small burr hole (1.5 mm) was placed in the skull for the insertion of a fiberoptic ICP transducer (Camino, San Diego, CA). Arterial blood gases and pH were periodically checked using an acid-base analyzer (Radiometer, Copenhagen, Denmark). Rectal temperature was monitored and maintained via an automatic thermal heating blanket (Harvard). No paralyzing drugs were used in any of these experiments. If apnea occurred and lasted more than 30 s during any of the experiments, the cat was attached to a small animal respirator (Harvard Apparatus, S. Natick, MA) and artificially ventilated. At the end of the experiment the cats were rapidly decapitated using a large animal decapitator (Harvard Apparatus).

C. BRAIN MISSILE WOUNDING PROCEDURE

Animals who received BMW had the anterior wall (1 cm x 1 cm) of the right frontal sinus was removed. The cats were wounded by a 2 mm having an energy of 2.4 J. The missile entered the tip of the right cerebral hemisphere and tracked posteriorly angling 20 degrees from the midline (9). A wound of this energy is considered a severe wound and would be expected to eventually cause fatal apnea in 70% of the animals (9). The Cushing response occurred within 5 s of wounding in all animals. All cats were sacrificed 6 mins after the initial brain injury. Controls were treated in the exact same manner, but were not injured.

D. TISSUE DISSECTION

After rapid decapitation, the cranium was opened, the brain removed and frozen by immersion in cold dichlorodifluoromethane (-40°C), then stored at -70°C. until the sampling process. The euthanization, brain removal and freezing procedure was kept as uniform as possible and took approximately 18 mins to complete for each animal. The brains were subsequently brought to approximately -15°C and sliced into 3-5 mm thick coronal sections with reference to standard cat brain atlases (2,61). Each brain was completely sliced in one session (10 min). Tissue samples were taken from still frozen slices with the aid of tissue micropunches (1-1.5 mm dia.) or a scalpel, placed in dry ice and then stored at -70°C until assayed (1-2 days). The brainstem was sliced in three 5 mm intervals, rostrally, starting at the obex. Two samples (left and right) of the NTS and area A1C1 were taken from the caudal slice. Two raphe nuclei samples were taken from the middle slice. Two raphe nuclei and two (left and right) LC samples were taken from the rostral slice. Hypothalamic slices were made by a three-bladed (3 mm interval) apparatus with the middle blade being positioned in the middle of the pituitary stalk. The anterior hypothalamus (AH) was dissected from the anterior slice by removing a 3 x 5 mm wide sample from the caudal midline. The posterior hypothalamus (PH) was sampled by removing a 4 x 4 x 4 mm triangular sample from the caudal midline of the posterior slice.

E. SAMPLE PREPARATION

For the determination of brain biogenic amines the left and right side samples of the NTS, area A1C1 and LC were analyzed individually, but the four raphe nuclei samples were analyzed as one sample. Tissue samples were homogenized by ultrasonic disruption in .05 M $HClO_4$ containing .5 mM EDTA and 20 pmol of NM as an internal standard. The volume of .05 M $HClO_4$ used was optimized from preliminary tests so that an adequate signal would be output to a strip chart recorder set at 20 nA/V full scale. The volume of .05 M $HClO_4$ were as follows: 200 ul for the NTS, area A1C1 and the LC, 300 ul for the raphe N.; 1000 ul for the AH and PH. Following centrifugation (40,000 x g, 25 min.), the supernatant was removed and refrigerated until injection into the HPLC; 20 ul was injected into the HPLC. The remaining tissue pellets were solubilized in .2 M NaOH and assayed for protein content using BCA* protein assay reagent (Pierce Chemical, Rockford, IL) and bovine serum albumin as a standard.

F. HIGH PRESSURE LIQUID CHROMATOGRAPHY

A BAS 200 HPLC system with an electrochemical detector was utilized for all analyses. Data was collected, integrated and quantified utilizing a PC-based chromatographic control system

(BAS PC-II). The analytical column used for all assays was a 100 mm x 3.1 mm, BAS Phase II, 3 um reverse phase column.

For brain biogenic amine analyses the column was kept at 40 °C during its operation. The mobile phase consisted of .13 M MCA with .67 mM EDTA, adjusted to pH 3.1 with 10 M NaOH and filtered through .45 um durapore filter (Millipore, Bedford MA.). SOS was added as the ion pairing reagent at 170 mg/l and CH_3CN was added to a final concentration of 2.5%. The mobile phase was pumped at 1 ml/min and kept at 35°C to prevent any possible outgassing. The detector potential was maintained at +800 mV vs. Ag/AgCl reference electrode. The strip chart recorder was set at 20 nA/V full scale. These chromatographic conditions permitted the routine quantification of NE, EPI, DA, 5-HT, DOPAC, HVA and 5-HIAA. within 18 mins.

G. DATA ANALYSIS

Digitized data were used to quantify each compound. The concentration of the brain biogenic amines were determined by comparing sample peak areas with peak areas obtained from a mixture of working standards. All concentrations were corrected by the NM recovery value for each sample and are expressed as ng/mg protein.

Initially, the brain biogenic amines values from the right and left side samples of the NTS, area A1C1 and the LC in the control and experimental groups were compared using ANOVA (SAS statistical package). The were no significant differences between the right and left side samples of any brain area in any group (all probabilities >.8). Therefore the right and left side sample values were averaged and used in further statistical analyses. Comparisons between the control and experimental groups were made using ANOVA (SAS statistical package).

RESULTS

The main objective is to determine if the effects of BMW on biogenic amines in the brain stem and hypothalamus were solely attributable to the accompanying increase in ICP. Therefore it is necessary to compare data we previously acquired from experiments in which the ICP was artificially increased, without wounding (see 1989 yearly report), with the data obtained from BMW experiments performed since then. The data from experiments in which the ICP was increased, without wounding, presented in the present report are re-presentations of data we previously reported in detail in our 1989 yearly report, while the BMW data are our new data. Data displaying the comparison, using group means, of the results of artificially increasing ICP, without wounding, vs. BMW are in Figs. 1-11 and Tables 1-9 (appendix). Individual data acquired from cats used in the BMW experiments are presented in separate tables in the appendix (Tables 10-18)

Individual data acquired from cats used in increased ICP, without wounding, experiments was presented in our 1989 yearly report.

Nineteen cats were used in BMW experiments but 3 cats were excluded from the study. There were ballistic technical problems with 2 cats and one cat was anomalous because the skull over the frontal cortex was absent.

The effect on MABP and CPP resulting from artificially-induced (unwounded) and BMW-induced (2.4 J) increases in ICP are displayed in Figs. 1 and 2 respectively, and Tables 1-3, 10-12. With either BMW, with its concomitant increase in ICP, or with the rapid rise in ICP caused by mock CSF infusion into the cisterna magna, there was an immediate Cushing response: an immediate (< 5 secs) increase in MABP. Adequate CPP (>40 mm Hg) was maintained in both groups. None of the cats in these experiments required respiratory support. No significant effects on systemic pressures occurred in control cats who were surgically prepared but were neither wounded nor had the ICP artificially increased (Figs. 3 and 4; Tables 1-3, 10-12).

Fig. 1. MABP-ICP-CPP RELATIONSHIP OF CATS WITH ARTIFICIALLY INCREASED ICP
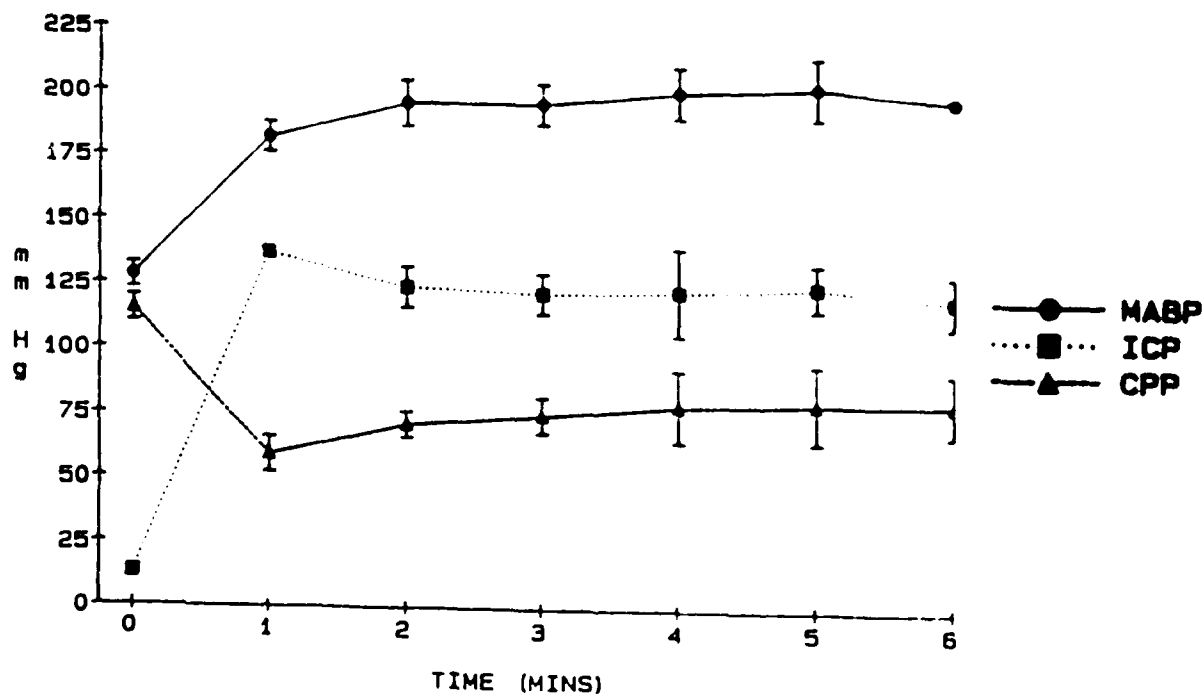
Values are Mean ± SEM (n=6)

Fig. 2. MABP-ICP-CPP RELATIONSHIP OF WOUNDED (BMW) CATS
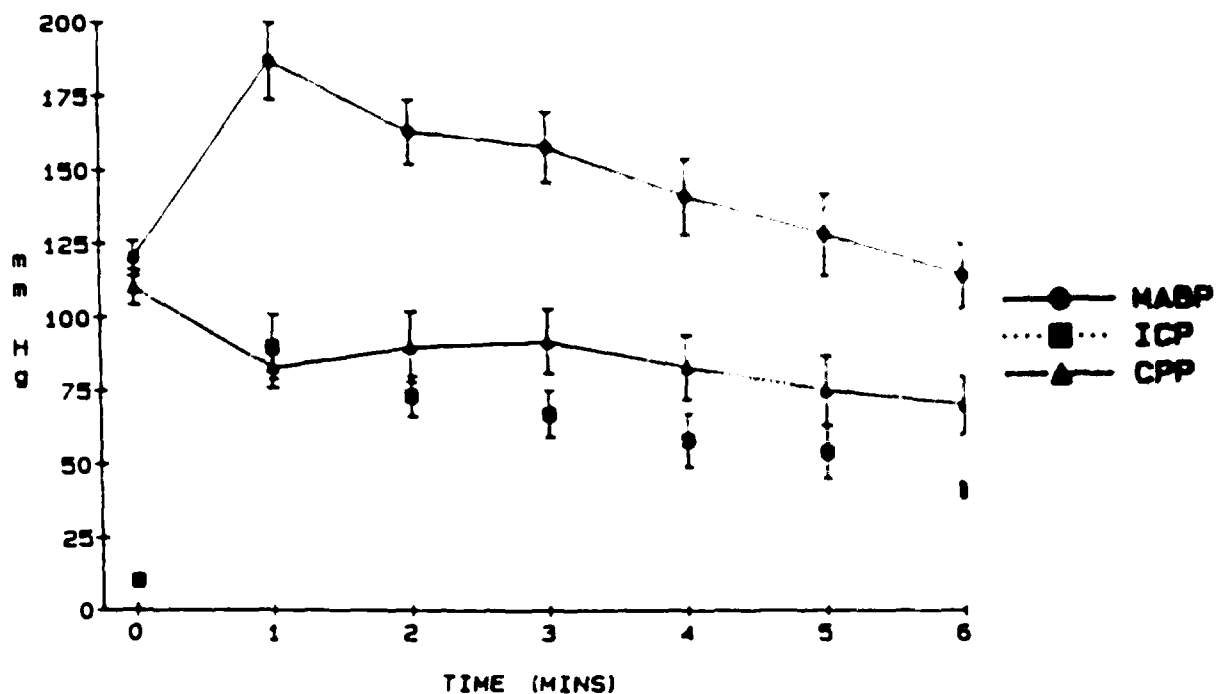
Values are Mean ± SEM (n=8)

Fig. 3. MABP-ICP-CPP RELATIONSHIP OF CONTROL CATS USED
IN ARTIFICIALLY INCREASED ICP EXPERIMENTS
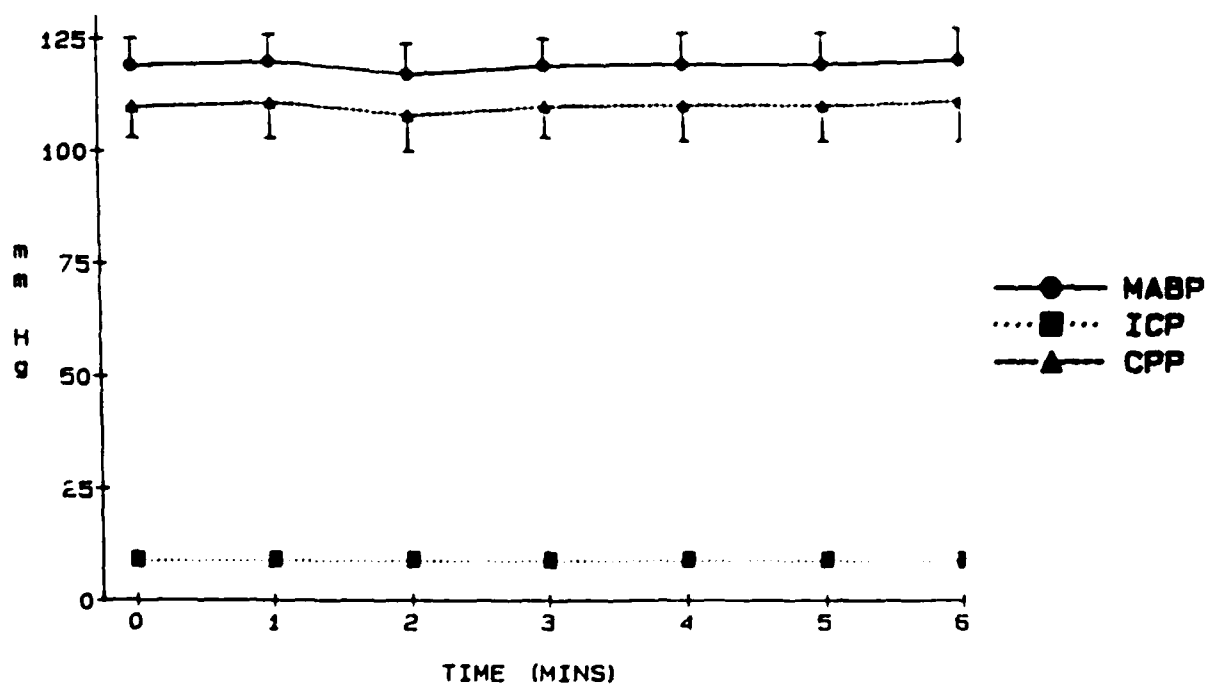
Values are Mean with SEM (n=6)

Fig. 4.   MABP-ICP-CPP RELATIONSHIP OF CONTROL CATS USED
          IN WOUNDING (BMW) EXPERIMENTS
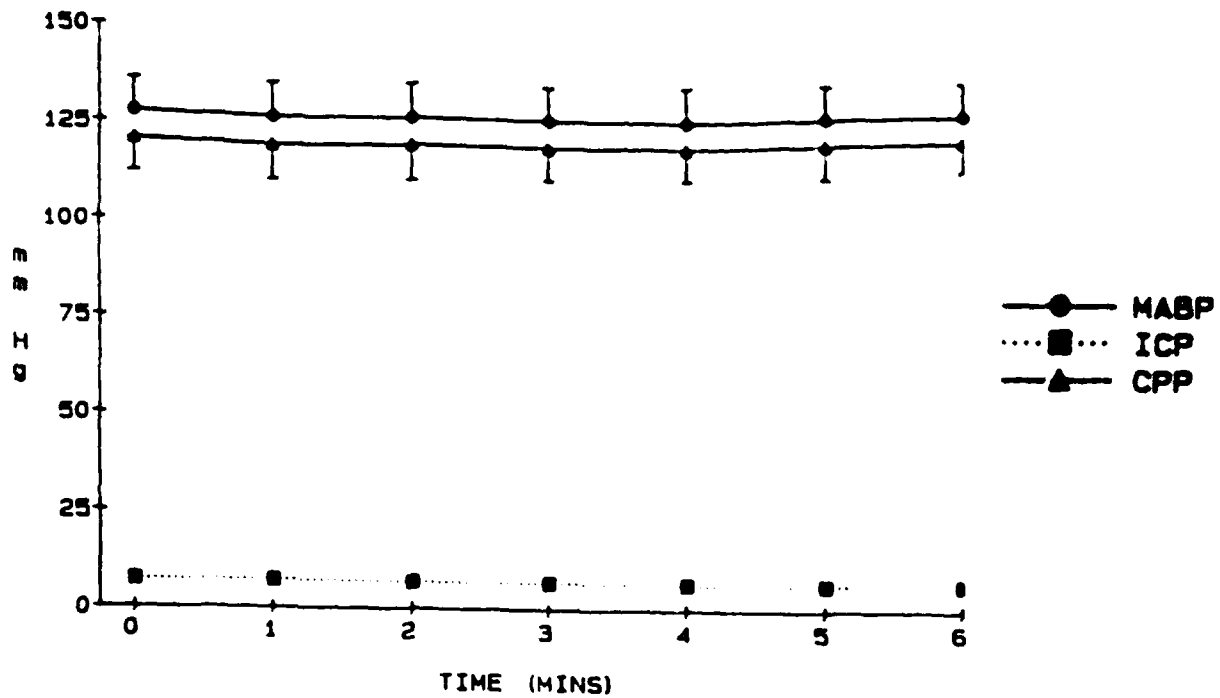
Values are Mean with SEM (n=8)

Significant and similar decreases in the EPI levels resulted
from both BMW- and artificially-induced increases in ICP in the
following brain areas: the NTS (60%, p<.001 and 57%, p<.01
respectively), area AlCl (74%, p<.001 and 68%, p<.001
respectively), the LC (60%, p<.001 and 66%, p<.01 respectively),
the raphe N. (56%, p<.001 and 47%. p<.05 respectively), and the
PH (63%, p<.001 and 66%, p<.01 respectively). EPI levels were
significantly decreased (56%, p<.001) in the AH only in wounded
cats (Fig. 5; Tables 4-9, 13-18).

Fig. 5.   COMPARISON OF THE EFFECTS OF ARTIFICIALLY INCREASED
           ICP (INC. ICP) AND BRAIN MISSILE WOUNDING (BMW) ON
           EPINEPHRINE (EPI) IN BRAIN STEM AREAS (NTS, AlCl, LC,
           RAPHE) AND THE HYPOTHALAMUS (AH, PH)
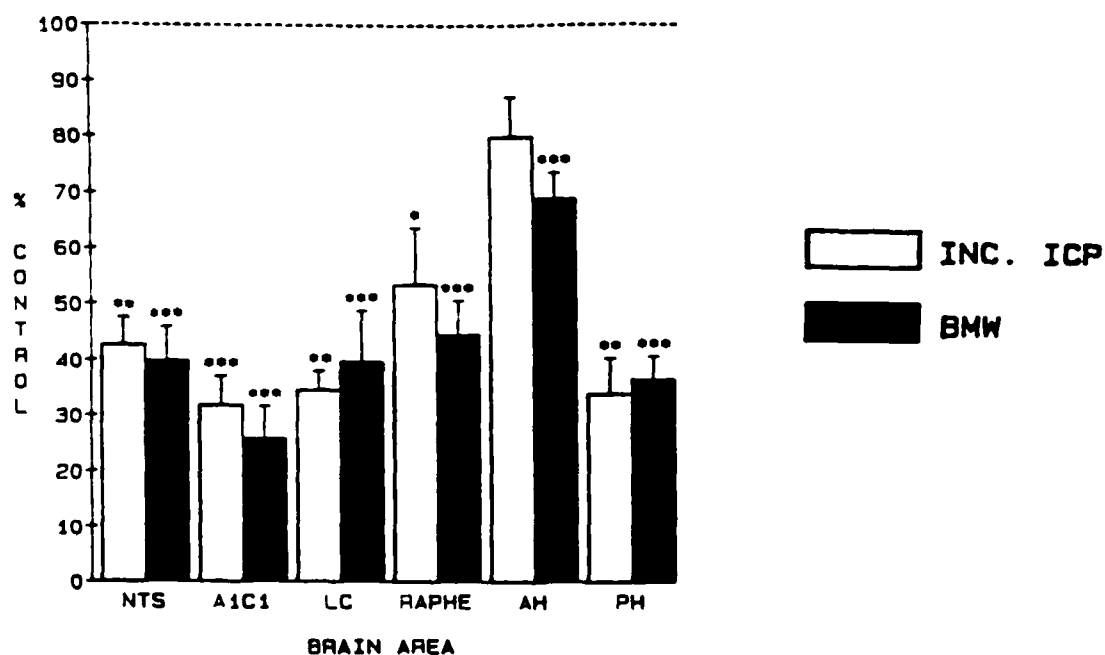
Values are Mean ± SEM (INC. ICP n=6, BMW n=8)
* p< .05, ** p< .01, *** p< .001

The NE levels were significantly decreased to approximately the same extent in the PH both from BMW- and artificially-induced increases in ICP (40%, p<.01 and 46%, p<.01 respectively). The wounded cats also had significant NE decreases in the NTS (21%, p<.01) and the area A1Cl (33%, p<.01) (Fig 6; Tables 4,5,9,13,14,18).

Fig. 6.   COMPARISON OF THE EFFECTS OF ARTIFICIALLY INCREASED ICP (INC. ICP) AND BRAIN MISSILE WOUNDING (BMW) ON NOREPINEPHRINE (NE) IN BRAIN STEM AREAS (NTS, A1Cl, LC, RAPHE) AND THE HYPOTHALAMUS (AH, PH)

Values are Mean ± SEM (INC. ICP n=6, BMW n=6)
** p< .01

The 5-HT and 5-HIAA levels were significantly decreased in the raphe N. both in the wounded cats (30%, p<.05; 22%, p<.05, respectively) and those with only artificially increased ICP (30%, p<.01; 36%, p<.01, respectively) (Figs. 7 & 8; Tables 7,16).

Fig. 7.   COMPARISON OF THE EFFECTS OF ARTIFICIALLY INCREASED ICP (INC. ICP) AND BRAIN MISSILE WOUNDING (BMW) ON SEROTONIN (5-HT) IN BRAIN STEM AREAS (NTS. A1C1, LC, RAPHE) AND THE HYPOTHALAMUS (AH, PH)

Values are Mean ± SEM (INC. ICP n=6, BMW n=8)
* p< .05, ** p< .01

Fig. 8. COMPARISON OF THE EFFECTS OF ARTIFICIALLY INCREASED
ICP (INC. ICP) AND BRAIN MISSILE WOUNDING (BMW) ON
5-HIAA IN BRAIN STEM AREAS (NTS, A1C1, LC, RAPHE) AND
THE HYPOTHALAMUS (AH, PH)
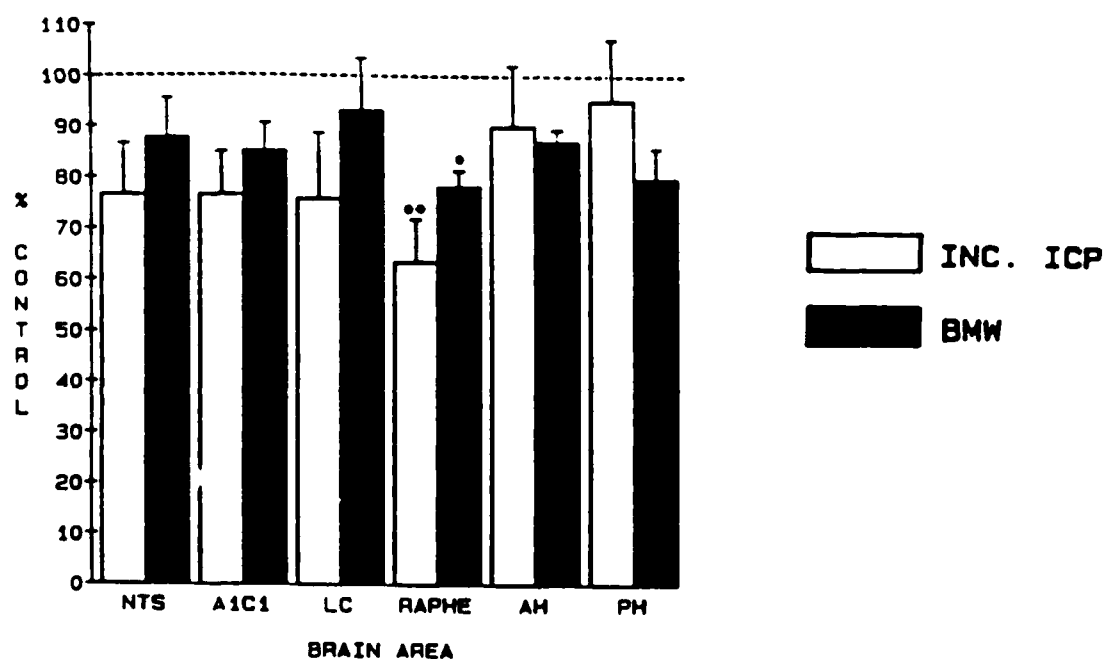
Values are Mean ± SEM (INC. ICP n=6, BMW n=8)
* p< .05, ** p< .01

Dopamine levels were significantly decreased in the raphe N. either from BMW- or artificially-induced increases in ICP (37%. p<.01 and 27%, p<.05, respectively). Significant decreases in dopamine in the NTS (39%, p<.05) and area A1C1 (48%, p<.05) occurred only in wounded cats (Fig. 9; Tables 1,5,7,13,15,16).

Concomitant decreases in HVA occurred in the same areas in which DA was decreased: the raphe N. of wounded cats (29%, p<.05) and those with only artificially increased ICP (32%, p<.01); the NTS (34%, p<.05) and area A1C1 (40%, p<.01) only in the wounded cats (Fig. 10; Tables 4,5,7,13,14,16). DOPAC levels were significantly decreased in the NTS (21%, p<.05) only in wounded cats (Fig. 11; Tables 4,13).

Fig. 9.   COMPARISON OF THE EFFECTS OF ARTIFICIALLY INCREASED ICP (INC. ICP) AND BRAIN MISSILE WOUNDING (BMW) ON DOPAMINE (DA) IN BRAIN STEM AREAS (NTS, A1C1, LC, RAPHE) AND THE HYPOTHALAMUS (AH, PH)
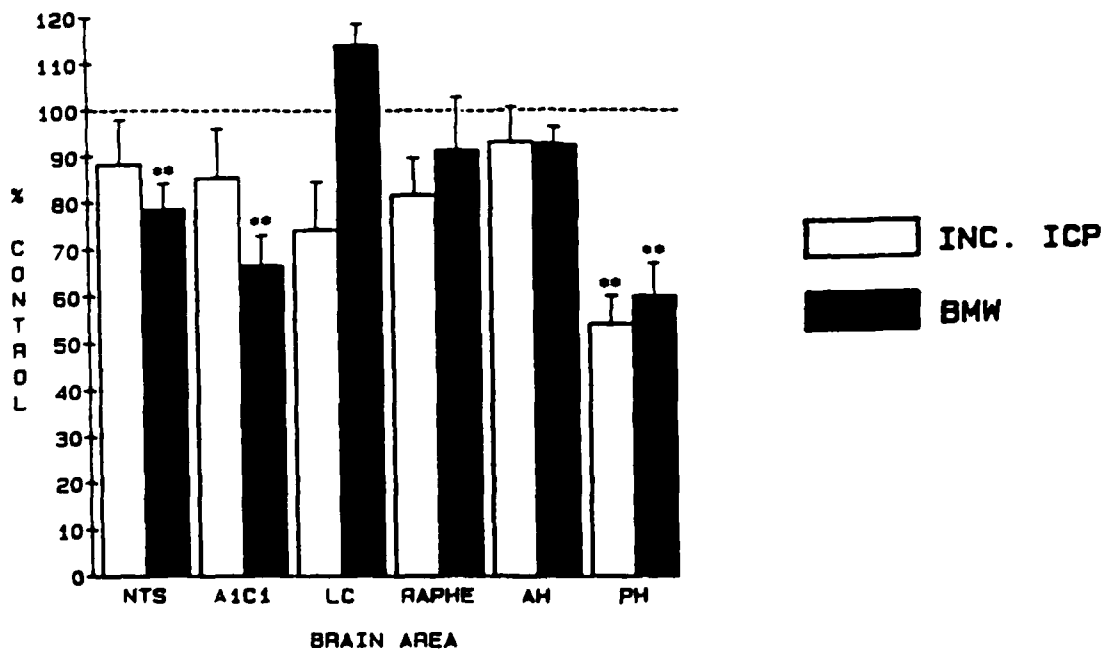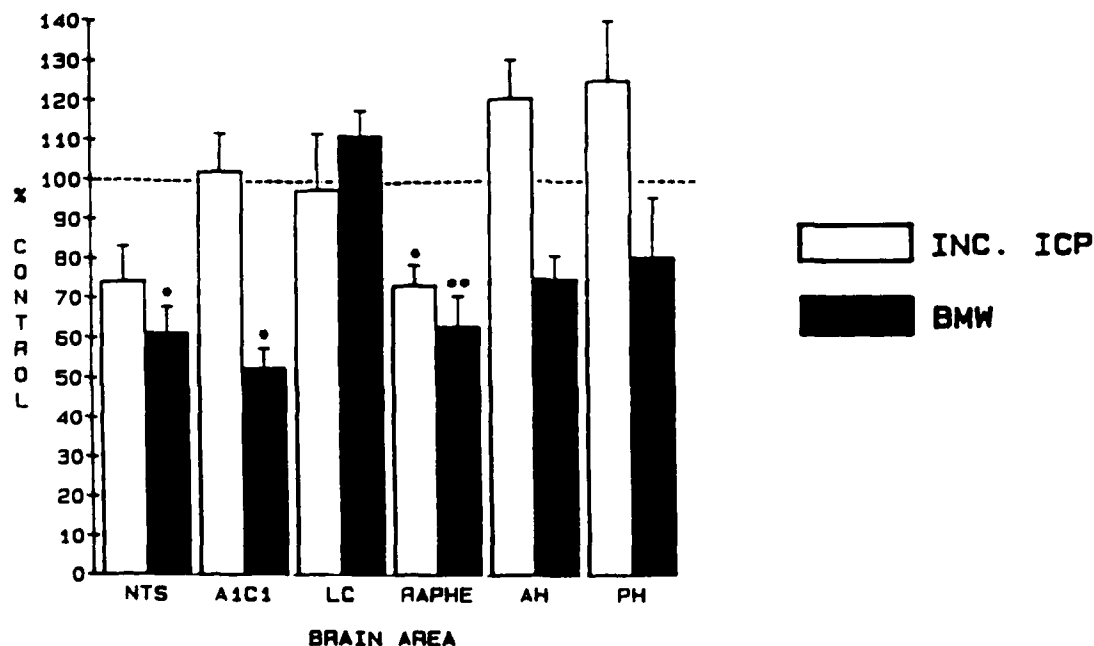

Values are Mean ± SEM (INC. ICP n=6, BMW n=8)
* p< .05, ** p< .01

Fig. 10. COMPARISON OF THE EFFECTS OF ARTIFICIALLY INCREASED
ICP (INC. ICP) AND BRAIN MISSILE WOUNDING (BMW) ON
HVA IN BRAIN STEM AREAS (NTS, A1C1, LC, RAPHE) AND THE
HYPOTHALAMUS (AH, PH)
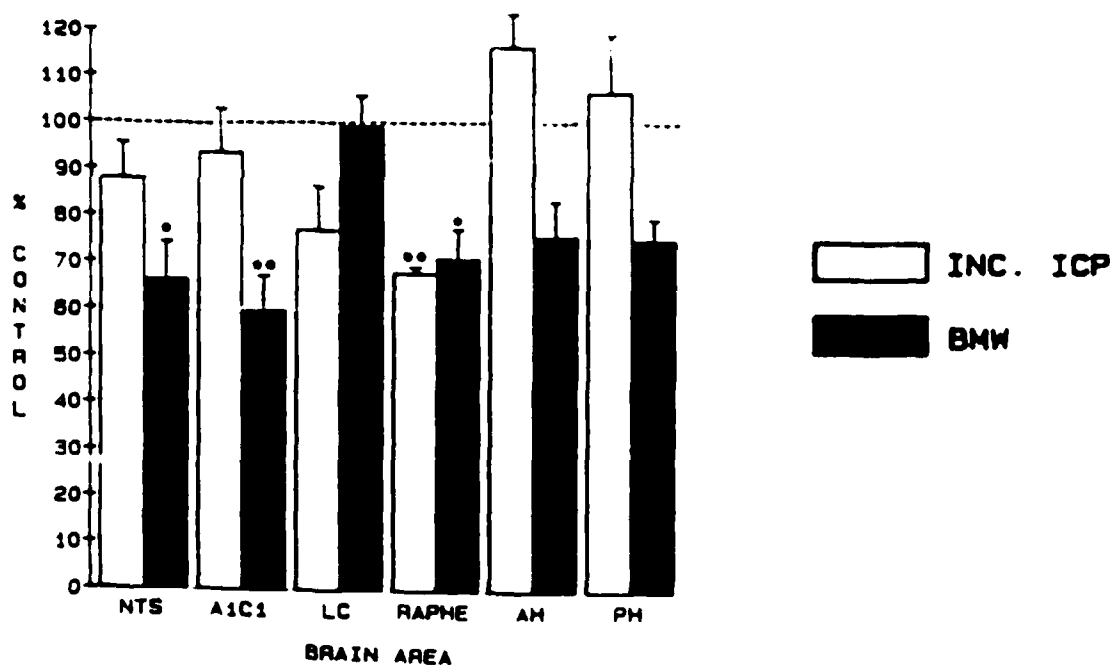
Values are Mean ± SEM (INC. ICP n=6, BMW n=8)
* p< .05, ** p< .01

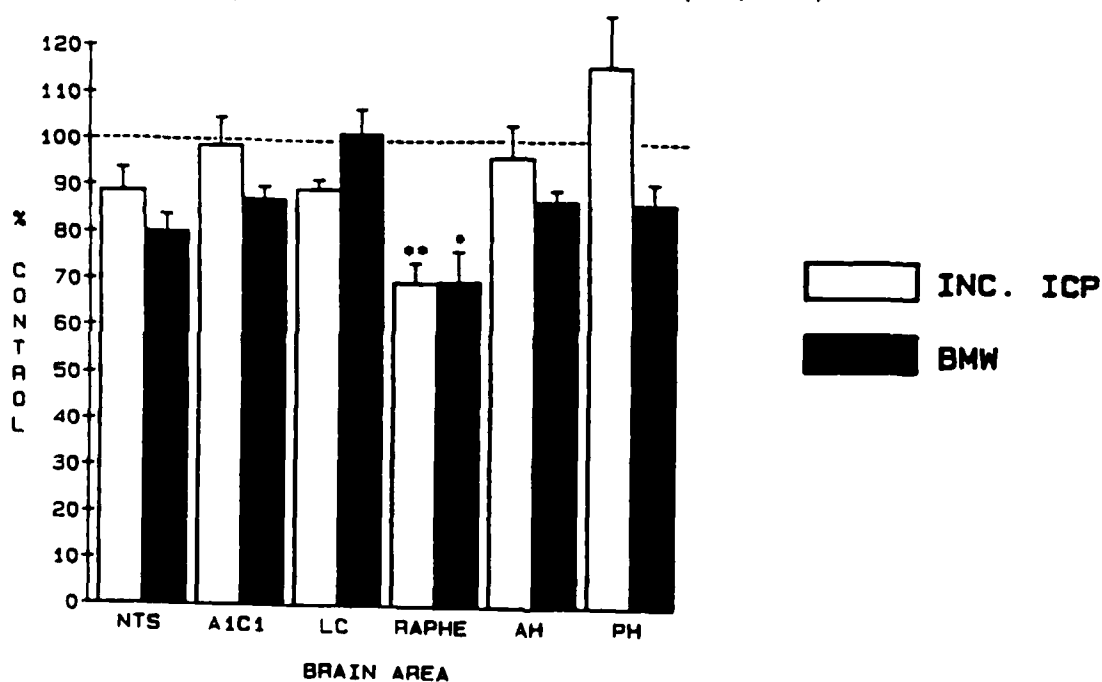Fig. 11. COMPARISON OF THE EFFECTS OF ARTIFICIALLY INCREASED
ICP (INC. ICP) AND BRAIN MISSILE WOUNDING (BMW) ON
DOPAC IN BRAIN STEM AREAS (NTS, A1C1, LC, RAPHE) AND
THE HYPOTHALAMUS (AH, PH)
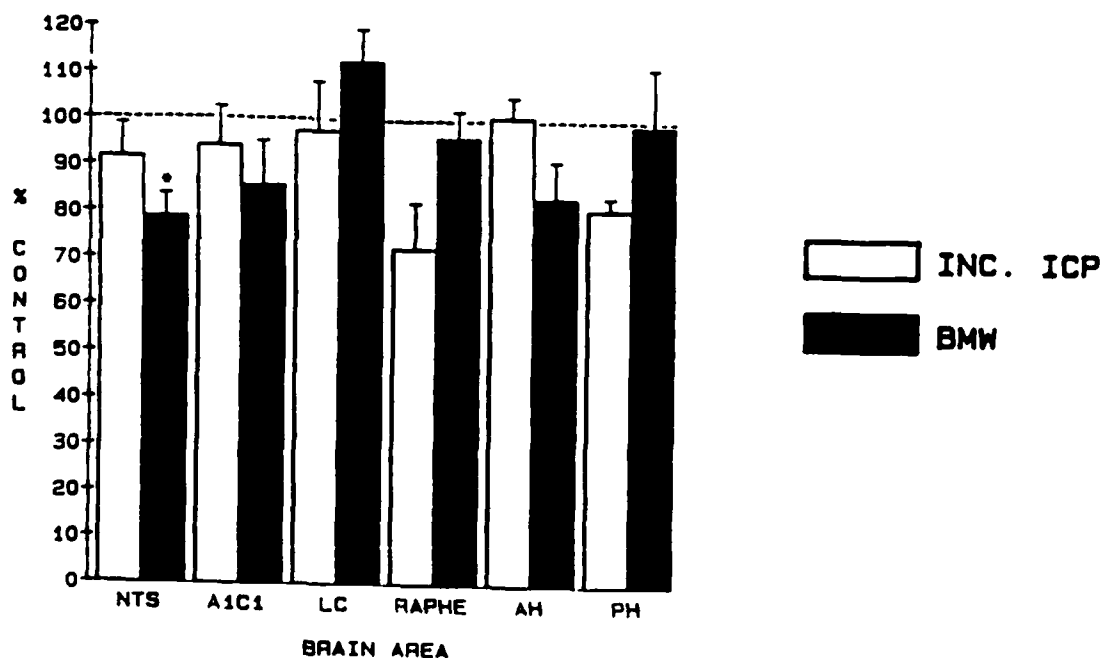
Values are Mean ± SEM (INC. ICP n=6, BMW n=8)
* p< .05

DISCUSSION

The present results compare the effects of brain wounding by a missile (BMW), with its concomitant rise in ICP, to an ICP increase alone on brain stem and hypothalamic monoamines. The artificial increase in ICP, without wounding, was induced by infusing mock CSF into the cisterna magna. An immediate Cushing response occurred following both experimental procedures. The Cushing response consists of an increase in MABP, bradycardia and general sympathetic nervous system activation presumably in response to increased ICP (12,13). The exact cause of the increased ICP which accompanies BMW is uncertain. Possibilities include intracranial bleeding, increased brain intravascular volume, increased cerebrospinal fluid (CSF) formation, or decreased CSF absorption. The effects of BMW or increased ICP alone on biogenic amines in discrete brain areas has not been previously reported.

We feel that biogenic amine changes observed in the brain stem and hypothalamus in these experiments did not occur secondary to ischemia because adequate CPP was maintained throughout the experimental period and other experiments in our laboratory indicate adequate cerebral blood flow to these areas (unpublished observations). Measurements of regional cerebral blood flow (microspheres) usually indicate no brain stem ischemia as a result of artificially-induced increases in ICP unless the CPP is very low (<40 mm Hg) (68). The brain stem is less susceptible to diminished CPP than higher cortical areas of the brain (38,56,68).

The most basic effect of increased ICP is an increase in sympathetic nerve activity which can be so massive that parasympathetic effects are overridden (30,34). Various methods have demonstrated that the characteristic cardiovascular (CV) responses to increased ICP and elicitation of the Cushing response are mediated by the sympathetic nervous system (SNS) (7,18,30,42,56).

Generalized activation of the SNS is also the main component of a stress response. Therefore, there is similarity between increased ICP- and stress-induced CV responses.

Wounded cats and those with only artificially increased ICP had substantial (47-68%) statistically significant decreases in EPI levels in the hypothalamus (PH and AH) and brain stem areas (NTS, area AlCl, LC, raphe N.) examined. The one exception was in the AH of unwounded cats with artificially increased ICP where EPI levels were reduced but did not reach statistical significance (Fig. 5). The similar magnitudes of EPI reductions in all other areas resulting from either BMW-induced or artificially increased ICP suggests that the reductions were possibility related to the CV effects caused by increased ICP. Brain stem EPI neurons appear to be physiologically heterogeneous and have been proposed to subserve vasopressor and vasodepressor functions (22,23,36,52,58).

Other studies using stress paradigms have reported significant reductions in EPI levels, along with a decrease in phenylethanolamine-N-methyltransferase activity, in discrete brain stem areas and the hypothalamus (32,54,55).

The levels of NE and DA and its metabolites did not show consistent changes parallel to those of EPI, suggesting a dissociation between the EPI systems and the NE and DA systems. NE, like EPI, was similarly decreased in the PH in response to both BMW- and artificially-induced increases in ICP. Conversely, there was no effect of BMW or artificially increased ICP on NE levels in the AH, raphe N. or LC, but EPI was reduced in all these areas. The NE and EPI depletions in the PH may be directly related to the vasopressor response to increased ICP. The PH and AH are thought to mediate vasopressor and vasodepressor responses, respectively (44) because electrical stimulation or application of NE or EPI directly to the PH or AH will increase or decrease MABP respectively (26,46,47,65).

Only BMW with its concomitant increase in ICP significantly reduced NE, DA and HVA levels in the NTS and area A1C1. Artificially increasing the ICP lowered the NE, DA and HVA levels in the NTS and area A1C1, but the decreases were not statistically significant. Since the levels of DA are small in the NTS and area A1C1, it is conceivable that the DA and HVA decreases are reflective of the NE decreases which occurred in these areas because DA is a precursor to NE in noradrenergic neurons. It is possible that the statistically significant decreases in NE in the NTS and area A1C1 result from the effects of brain wounding with very high instantaneous overpressures, and resultant pressure waves within the cranial vault. The NE decreases could also result from a more severe stress response to BMW-induced increased ICP but such a response was not reflected in the systemic pressures (Figs. 1 & 2). If anything, the artificially-induced increased ICP might be considered more stressful because a high ICP and MABP were maintained for a longer period of time.

5-HT and its metabolite 5-HIAA were significantly reduced only in the raphe N. both in response to both BMW and artificially increased ICP, which suggest that decreased serotonergic functioning in the raphe nuclei may be solely as a result of increased ICP. DA and HVA were also decreased about equally in the raphe nuclei with both BMW and artificially increased ICP. It is difficult to speculate on the meaning of the 5-HT and DA reductions in the raphe N., as relates to CV functions, since discrete raphe nuclei were not evaluated.

The overall monoamine profile of BMW and increased ICP suggests that the monoamine effects in the brain stem and hypothalamus are probably attributable to increased ICP alone. Artificially increasing the ICP and BMW both had generally the same effect on the brain biogenic amines. Artificially increasing the ICP via cisterna magna infusions of mock CSF is probably associated with minimal brain stem distortion since the

intracranial pressure is distributed equally throughout the brain. BMW and associated overpressures and increased ICP may lead to brain stem distortion since the pressure is quickly applied to the lateral supratentorial compartment. The possible brain stem distortion from BMW did not give any different results from artificially increased ICP except possibly on NE in the NTS and area A1C1 at 6 mins post-injury.

The CV effects of increased ICP are similar to that of a stress response. Previous studies using footshock or immobilization stress in conscious rats have revealed similar results in many of the brain areas presently examined, especially in regards to the adrenergic system (11,20,32,53,54,55,59). Similar results have also been found in the hypothalamus and brain stem using a hindlimb ischemia model in anesthetized rats (62,63). Concussive head injury in unanesthetized rats caused a DA increase in the pons-medulla area which was attributable to injury, but other significant monoamine alterations were found to be stress related because unanesthetized rats were used and ICP alterations were also not accounted for (25).

The present EPI and NE data are not sufficient to conclusively determine if the depletions were due to increased EPI or NE utilization or decreased neuronal functioning. Therefore, whether the changes represent causative factors in the increase in MABP (vasopressor) or compensatory factors (vasodepressor) cannot be speculated upon.

It is important to note that despite brain injury and/or large increases in ICP all monoamines were not depleted 6 mins post-injury indicating that, at least at this time point, some maintenance of the integrity of the brain stem monoaminergic systems despite rather severe perturbations. A total, across the board depletion may have been indicative of severe brainstem disruption and/or widespread cell death. Our results suggest that even at a wound energy expected to be fatal 70% of the time (9) or at very high ICP levels, initially, selective, organized alterations occurred in the EPI systems overall and 5-HT system in the raphe N. Whether a more prolonged duration of high ICP would preferentially deplete the EPI and 5-HT systems even further is presently unknown as are the effects of BMW on brain stem monoamines beyond 6 mins after injury. Possibly, EPI and/or 5-HT depletions could be deleterious in the face of further severe physiological stress such as high ICP and play a role in eventual CV and/or respiratory failure in brain injury. If so, these systemic effects may be amenable to pharmacological intervention.

GENERAL SIGNIFICANCE

The effects of brain missile wounding (BMW), with its accompanying increase in ICP and instantaneous overpressures, had not been previously evaluated and it was unknown whether BMW led to any overall or generalized effects on brain stem monoaminergic

functioning. Overall effects or depletion of all monoamine systems may suggest a severe dysfunction of brain stem functions indicating possible cellular disruption or death.

In order to discern the effects of BMW, with its accompanying increase in ICP, from the effects of increased ICP alone, the results of BMW were compared to the results of increased ICP alone, without wounding (1989 yearly report). This comparison indicated that neither BMW nor increased ICP alone led to global effects, an important finding. The comparison further indicated that the effects on brain stem monoamines were selective and similar: depletions of EPI overall and decreased functioning of 5-HT and DA in the raphe nuclei. BMW, however, did result in additional decreases of NE in the NTS and area AlC1. From this comparison we concluded that the effects of BMW on brain stem monoamines are probably the result of the stress of increased ICP, i.e. stress-induced cardiovascular changes.

The present study on brain stem monoamines focused on effects at 6 mins. post-injury. Severe depletions (47%-68%) of EPI were found in every brain stem area examined. The effects on EPI systems at longer time periods post-injury is unknown. It is possible that a longer period of prolonged stress could deplete the EPI further and be causally related to later cardiovascular and/or respiratory dysfunction. If so, it may be possible to use selective pharmacological agents which may prevent the compromise or at least maintain the EPI systems until more appropriate action can be delivered. It is also possible that the depletion of the EPI systems are reflective of depletions of suspected epinephrine co-transmitters (e.g. neuropeptide Y and substance P). The answers to these questions must await further study.

APPENDIX

Table 1. COMPARISON OF THE EFFECTS OF ARTIFICIALLY INCREASED INTRACRANIAL PRESSURE (INC. ICP) AND BRAIN MISSILE WOUNDING (BMW) ON MEAN ARTERIAL BLOOD PRESSURE (MABP)

	CONT	1 Min	2 Min	3 Min	4 Min	5 Min	6 Min
CONTROLS (n=6)							
MEAN	119	120	117	119	119	119	120
+/-SEM	6	6	7	6	7	7	7
INC. ICP (n=6)							
MEAN	128	182	196	196	201	203	198
+/-SEM	5	6	9	8	10	12	1
CONTROLS (n=8)							
MEAN	127	126	126	125	125	126	127
+/-SEM	9	9	9	9	9	9	9
BMW (n=8)							
MEAN	120	187	163	158	141	128	113
+/-SEM	6	13	11	12	13	14	11

Values are mm Hg

Table 2. COMPARISON OF THE EFFECTS OF ARTIFICIALLY INCREASED INTRACRANIAL PRESSURE (INC. ICP) AND BRAIN MISSILE WOUNDING (BMW) ON MEAN INTRACRANIAL PRESSURE (ICP)

	CONT	1 Min	2 Min	3 Min	4 Min	5 Min	6 Min
CONTROLS (n=6)							
MEAN	9	9	9	9	9	9	9
+/-SEM	2	2	2	2	2	2	2
INC. ICP (n=6)							
MEAN	13	137	124	122	123	125	120
+/-SEM	1	2	8	8	17	9	10
CONTROLS (n=8)							
MEAN	7	7	7	7	7	7	7
+/-SEM	1	1	1	1	1	1	1
BMW (n=8)							
MEAN	10	91	73	67	58	53	43
+/-SEM	1	11	7	8	9	9	2

Values are mm Hg

Table 3. COMPARISON OF THE EFFECTS OF ARTIFICIALLY INCREASED INTRACRANIAL PRESSURE (INC. ICP) AND BRAIN MISSILE WOUNDING (BMW) ON CEREBRAL PERFUSION PRESSURE (CPP)

	CONT	1 Min	2 Min	3 Min	4 Min	5 Min	6 Min
CONTROLS (n=6)							
MEAN	110	111	108	110	110	110	111
+/-SEM	7	8	8	7	8	8	9
INC. ICP (n=6)							
MEAN	115	59	71	75	79	80	80
+/-SEM	5	7	5	7	14	15	12
CONTROLS (n=8)							
MEAN	120	119	119	118	118	119	121
+/-SEM	8	9	9	8	8	8	8
BMW (n=8)							
MEAN	110	83	90	92	83	75	70
+/-SEM	6	7	12	11	11	12	10

Values are mm Hg

Table 4. COMPARISON OF THE EFFECTS OF ARTIFICIALLY INCREASED INTRACRANIAL PRESSURE (INC. ICP) AND BRAIN MISSILE WOUNDING (BMW) ON BIOGENIC AMINES AND METABOLITES IN THE NUCLEUS TRACTUS SOLITARIUS (NTS)

	NE	EPI	DA	DOPAC	HVA	5-HT	5-HIAA
CONTROL (n=6)							
MEAN	12.927	0.982	0.864	0.743	1.986	21.623	12.607
+/-SEM	1.298	0.049	0.118	0.036	0.117	1.061	1.631
INC. ICP (n=6)							
MEAN	11.415	0.420**	0.640	0.682	1.748	19.191	9.668
+/-SEM	1.259	0.048	0.076	0.054	0.147	1.036	1.244
CONTROL (n=8)							
MEAN	16.247	0.862	0.945	0.585	2.154	23.746	10.266
+/-SEM	0.796	0.080	0.143	0.039	0.233	1.414	0.783
BMW (n=8)							
MEAN	12.790**	0.344***	0.577*	0.462*	1.424*	19.057	9.024
+/-SEM	0.906	0.052	0.062	0.029	0.174	0.869	0.798

Values are ng/mg protein
* p<.05, ** p<.01, *** p<.001

Table 5.   COMPARISON OF THE EFFECTS OF ARTIFICIALLY
           INCREASED INTRACRANIAL PRESSURE (INC. ICP) AND
           BRAIN MISSILE WOUNDING (BMW) ON BIOGENIC AMINES
           AND METABOLITES IN AREA A1C1

	NE	EPI	DA	DOPAC	HVA	5-HT	5-HIAA
CONTROL (n=6)							
MEAN	9.451	0.849	0.591	0.850	1.772	17.060	12.930
+/-SEM	0.808	0.038	0.018	0.108	0.071	0.542	1.642
INC. ICP (n=6)							
MEAN	8.084	0.269**	0.603	0.804	1.657	16.834	9.935
+/-SEM	1.002	0.045	0.057	0.071	0.164	1.044	1.091
CONTROL (n=8)							
MEAN	12.119	0.801	0.795	0.602	2.156	18.288	11.347
+/-SEM	0.917	0.089	0.127	0.056	0.187	1.005	0.677
BMW (n=8)							
MEAN	8.101**	0.206***	0.416*	0.517	1.289***	15.970	9.684
+/-SEM	0.761	0.046	0.039	0.058	0.160	0.518	0.641

Values are ng/mg protein
* p<.05, ** p<.01, *** p<.001

Table 6.   COMPARISON OF THE EFFECTS OF ARTIFICIALLY
           INCREASED INTRACRANIAL PRESSURE (INC. ICP) AND
           BRAIN MISSILE WOUNDING (BMW) ON BIOGENIC AMINES
           AND METABOLITES IN THE LOCUS COERULEUS (LC)

	NE	EPI	DA	DOPAC	HVA	5-HT	5-HIAA
CONTROL (n=6)							
MEAN	22.437	1.061	1.871	4.190	15.336	25.578	29.630
+/-SEM	1.971	0.167	0.217	0.186	0.984	0.681	2.556
INC. ICP (n=6)							
MEAN	16.668	0.366**	1.821	4.096	11.823	22.875	22.524
+/-SEM	2.288	0.037	0.267	0.862	1.434	0.506	3.828
CONTROL (n=8)							
MEAN	19.323	0.735	1.430	3.426	11.215	21.013	19.991
+/-SEM	1.281	0.070	0.100	0.437	0.749	1.053	1.757
BMW (n=8)							
MEAN	22.101	0.291***	1.586	3.855	11.119	21.320	18.632
+/-SEM	0.888	0.066	0.093	0.238	0.756	1.130	2.078

Values are ng/mg protein
** p<.01, *** p<.001

Table 7. COMPARISON OF THE EFFECTS OF ARTIFICIALLY
INCREASED INTRACRANIAL PRESSURE (INC. ICP) AND
BRAIN MISSILE WOUNDING (BMW) ON BIOGENIC AMINES
AND METABOLITES IN THE RAPHE NUCLEI

	NE	EPI	DA	DOPAC	HVA	5-HT	5-HIAA
CONTROL (n=6)							
MEAN	13.174	0.893	0.816	0.371	4.536	23.962	32.608
+/-SEM	0.915	0.224	0.079	0.048	0.246	1.870	2.457
INC. ICP (n=6)							
MEAN	10.767	0.475*	0.598*	0.287	3.087**	16.700**	20.713**
+/-SEM	1.050	0.117	0.041	0.037	0.054	1.060	2.737
CONTROL (n=8)							
MEAN	14.676	0.895	0.750	0.264	3.571	22.028	25.058
+/-SEM	0.940	0.043	0.046	0.019	0.384	2.455	2.028
BMW (n=8)							
MEAN	13.408	0.398***	0.470**	0.254	2.540*	15.471*	19.595*
+/-SEM	1.692	0.054	0.058	0.015	0.234	1.435	0.795

Values are ng/mg protein
* p<.05, ** p<.01, *** p<.001

Table 8. COMPARISON OF THE EFFECTS OF ARTIFICIALLY
INCREASED INTRACRANIAL PRESSURE (INC. ICP) AND
BRAIN MISSILE WOUNDING (BMW) ON BIOGENIC AMINES
AND METABOLITES IN THE ANTERIORHYPOTHALAMUS (AH)

	NE	EPI	DA	DOPAC	HVA	5-HT	5-HIAA
CONTROL (n=6)							
MEAN	48.833	3.289	3.736	1.430	8.264	19.845	13.800
+/-SEM	2.707	0.355	0.377	0.165	0.619	1.593	1.091
INC. ICP (n=6)							
MEAN	40.856	2.626	4.465	1.442	9.663	19.197	12.416
+/-SEM	3.338	0.238	0.360	0.061	1.403	1.398	1.659
CONTROL (n=8)							
MEAN	42.020	3.191	5.538	1.457	9.772	24.398	13.630
+/-SEM	2.134	0.216	0.732	0.146	0.877	1.390	0.889
BMW (n=8)							
MEAN	39.001	2.199***	4.416	1.214	7.420	21.329	11.839
+/-SEM	1.553	0.147	0.338	0.116	0.752	0.550	0.333

Values are ng/mg protein
*** p<.001

Table 9.   COMPARISON OF THE EFFECTS OF ARTIFICIALLY
INCREASED INTRACRANIAL PRESSURE (INC. ICP) AND
BRAIN MISSILE WOUNDING (BMW) ON BIOGENIC AMINES
AND METABOLITES IN THE POSTERIOR HYPOTHALAMUS (PH)

	NE	EPI	DA	DOPAC	HVA	5-HT	5-HIAA
CONTROL (n=6)							
MEAN	40.757	2.720	2.484	1.609	5.969	13.242	9.461
+/-SEM	3.783	0.425	0.205	0.142	0.449	1.481	0.653
INC. ICP (n=6)							
MEAN	22.085**	0.922**	3.153	1.311	6.390	15.412	8.999
+/-SEM	2.504	0.177	0.328	0.041	0.750	1.044	1.137
CONTROL (n=8)							
MEAN	44.569	2.421	6.172	2.120	6.768	19.963	12.626
+/-SEM	3.622	0.240	1.345	0.350	0.789	0.891	1.089
BMW (n=8)							
MEAN	26.848**	0.888***	2.957	2.099	5.092	17.332	10.048
+/-SEM	3.027	0.103	0.785	0.689	0.308	0.881	0.746

Values are ng/mg protein
** p<.01, *** p<.001

Table 10.   EFFECT OF BRAIN MISSILE WOUNDING (BMW) ON MEAN
ARTERIAL BLOOD PRESSURE (MABP)

	CONT	1 Min	2 Min	3 Min	4 Min	5 Min	6 Min
CONTROLS							
J2	86	82	82	91	88	87	85
J5	109	109	109	109	109	109	116
J8	117	117	117	118	117	123	120
J11	134	136	136	131	131	131	132
J13	163	163	163	163	163	163	163
J14	117	117	117	117	117	123	127
J15	137	127	127	117	117	117	120
J16	157	157	157	157	157	157	157
MEAN	127	126	126	125	125	126	127
+/-SEM	9	9	9	9	9	9	9
BMW							
J1	127	156	133	140	123	123	113
J3	137	210	152	145	117	102	102
J4	113	243	221	213	193	185	163
J6	100	146	135	104	79	80	77
J7	110	147	137	166	155	145	138
J9	118	194	158	135	125	75	75
J10	105	168	178	167	162	140	140
J12	152	198	186	197	174	174	100
MEAN	120	187	163	158	141	128	113
+/-SEM	6	13	11	12	13	14	11

Values are mm Hg

Table 11. EFFECT OF BRAIN MISSILE WOUNDING (BMW) ON INTRACRANIAL PRESSURE (ICP)

	CONT	1 Min	2 Min	3 Min	4 Min	5 Min	6 Min
CONTROLS							
J2	5	5	5	5	5	5	5
J5	7	7	7	7	7	7	7
J8	9	9	7	7	5	5	5
J11	5	5	5	5	5	5	5
J13	9	9	9	9	9	9	9
J14	5	5	5	5	5	5	5
J15	6	6	6	6	6	6	6
J16	12	12	12	12	12	12	12
MEAN	7	7	7	7	7	7	7
+/-SEM	1	1	1	1	1	1	1
BMW							
J1	15	100	79	63	50	42	35
J3	12	136	81	74	50	45	38
J4	10	89	72	68	62	50	50
J6	6	78	78	58	44	44	44
J7	8	50	50	43	43	43	45
J9	14	87	56	56	48	40	40
J10	10	61	52	52	52	48	48
J12	7	130	115	119	116	116	46
MEAN	10	91	73	67	58	53	43
+/-SEM	1	11	7	8	9	9	2

Values are mm Hg

Table 12. EFFECT OF BRAIN MISSILE WOUNDING (BMW) ON CEREBRAL PERFUSION PRESSURE (CPP)

	CONT	1 Min	2 Min	3 Min	4 Min	5 Min	6 Min
CONTROLS							
J2	81	77	77	86	83	82	79
J5	102	102	102	102	102	102	109
J8	108	108	110	111	112	118	115
J11	129	131	131	126	126	126	127
J13	154	154	154	154	154	154	154
J14	112	112	112	112	112	118	122
J15	131	121	121	111	111	111	114
J16	145	145	145	145	145	145	145
MEAN	120	119	119	118	118	119	121
+/-SEM	8	9	9	8	8	8	8
BMW							
J1	112	56	54	77	73	81	78
J3	115	74	71	71	67	57	64
J4	103	77	149	145	131	135	113
J6	94	75	57	46	35	36	33
J7	102	97	87	123	112	102	93
J9	114	107	102	82	77	35	35
J10	95	107	126	115	110	92	92
J12	145	68	71	78	58	58	54
MEAN	110	83	90	92	83	75	70
+/-SEM	6	7	12	11	11	12	10

Values are mm Hg

Table 13. **EFFECT OF BRAIN MISSILE WOUNDING (BMW) ON BIOGENIC AMINES AND METABOLITES IN THE NUCLEUS TRACTUS SOLITARIUS (NTS)**

	NE	EPI	DA	DOPAC	HVA	5-HT	5-HIAA
CONTROLS							
J2	15.540	0.439	0.600	0.486	2.204	27.003	11.294
J5	13.846	0.698	0.436	0.795	1.550	17.857	9.304
J8	20.119	0.706	0.541	0.467	1.408	17.5¯6	6.928
J11	17.261	0.956	0.890	0.587	1.341	26.938	8.492
J13	14.740	1.071	1.158	0.476	2.579	25.822	13.807
J14	17.400	1.086	1.225	0.613	2.755	27.460	11.651
J15	17.748	1.027	1.614	0.603	2.286	23.267	8.992
J16	13.538	0.913	1.096	0.653	3.106	24.043	11.662
MEAN	16.247	0.862	0.945	0.585	2.154	23.746	10.266
+/-SEM	0.796	0.080	0.143	0.039	0.233	1.414	0.783
BMW							
J1	13.432	0.211	0.569	0.427	2.131	20.557	12.556
J3	13.975	0.234	0.625	0.493	2.035	19.034	10.294
J4	7.900	0.194	0.291	0.349	0.687	14.503	6.370
J6	13.829	0.215	0.583	0.567	1.276	18.758	7.971
J7	16.453	0.473	0.914	0.561	1.645	22.390	8.991
J9	11.386	0.461	0.549	0.414	1.409	17.661	8.030
J10	14.051	0.564	0.636	0.376	1.053	21.379	6.503
J12	11.294	0.403	0.449	0.507	1.153	18.178	11.480
MEAN	12.790**	0.344***	0.577*	0.462*	1.424*	19.057	9.024
+/-SEM	0.906	0.052	0.062	0.029	0.174	0.869	0.798

Values are ng/mg protein
* p<.05, ** p<.01, *** p<.001

Table 14. **EFFECT OF BRAIN MISSILE WOUNDING (BMW) ON BIOGENIC AMINES AND METABOLITES IN AREA A1C1**

	NE	EPI	DA	DOPAC	HVA	5-HT	5-HIAA
CONTROL							
J2	10.317	0.477	0.466	0.539	2.508	12.350	13.665
J5	13.392	0.640	0.488	0.607	1.576	19.761	10.053
J8	16.689	0.693	0.626	0.854	2.127	18.858	13.474
J11	8.667	0.834	0.455	0.309	1.265	17.149	8.448
J13	14.386	1.286	1.262	0.715	2.783	21.330	13.123
J14	11.320	1.026	0.715	0.523	2.111	16.932	11.150
J15	11.971	0.761	1.337	0.600	2.172	19.307	9.784
J16	10.212	0.688	1.007	0.671	2.703	20.616	11.076
MEAN	12.119	0.801	0.795	0.602	2.156	18.288	11.347
+/-SEM	0.917	0.089	0.127	0.056	0.187	1.005	0.677
BMW							
J1	10.379	0.083	0.540	0.661	2.056	18.225	11.967
J3	10.863	0.173	0.614	0.697	1.741	16.934	10.823
J4	9.118	0.229	0.373	0.488	0.786	14.947	8.764
J6	9.270	0.091	0.403	0.501	1.273	15.587	8.337
J7	6.840	0.079	0.446	0.614	1.536	13.983	9.024
J9	5.512	0.212	0.303	0.163	1.033	15.620	8.763
J10	5.144	0.442	0.312	0.521	0.846	16.049	7.402
J12	7.683	0.338	0.339	0.491	1.037	17.001	12.396
MEAN	8.101**	0.206***	0.416*	0.517	1.289**	15.970	9.684
+/-SEM	0.761	0.046	0.039	0.058	0.160	0.518	0.641

Values are ng/mg protein
* p<.05, ** p<.01, *** p<.001

Table 15. EFFECT OF BRAIN MISSILE WOUNDING (BMW) ON BIOGENIC AMINES AND METABOLITES IN THE LOCUS COERULEUS (LC)

	NE	EPI	DA	DOPAC	HVA	5-HT	5-HIAA
CONTROL							
J2	23.583	0.380	1.395	2.897	13.162	22.228	21.876
J5	16.366	0.908	1.637	2.917	14.488	20.793	30.932
J8	19.450	0.468	1.029	2.614	9.126	15.319	20.469
J11	18.471	0.801	1.163	2.317	11.735	21.528	15.828
J13	17.537	0.832	1.488	1.800	8.533	24.607	20.257
J14	16.116	0.862	1.311	1.938	9.906	21.055	17.148
J15	17.004	0.761	1.948	1.897	10.090	18.561	15.624
J16	26.062	0.866	1.465	2.025	12.678	24.012	17.790
MEAN	19.323	0.735	1.430	3.426	11.215	21.013	19.991
+/-SEM	1.281	0.070	0.100	0.437	0.749	1.053	1.757
BMW							
J1	18.080	0.415	1.105	2.845	14.255	18.965	27.480
J3	21.715	0.222	1.655	4.153	11.583	21.020	21.899
J5	20.757	0.053	1.591	5.048	12.989	21.574	16.230
J6	22.724	0.107	1.470	3.542	10.926	17.176	12.659
J7	22.870	0.128	1.881	3.331	8.099	20.723	18.756
J9	27.005	0.513	1.952	4.363	11.587	28.244	16.173
J10	22.480	0.526	1.484	3.791	8.140	21.050	10.705
J12	21.175	0.360	1.550	3.765	11.536	21.810	25.153
MEAN	22.101	0.291***	1.586	3.855	11.119	21.320	18.632
+/-SEM	0.888	0.066	0.093	0.238	0.756	1.130	2.078

Values are ng/mg protein
*** p<.001

Table 16. EFFECT OF BRAIN MISSILE WOUNDING (BMW) ON BIOGENIC AMINES AND METABOLITES IN THE RAPHE NUCLEI

	NE	EPI	DA	DOPAC	HVA	5-HT	5-HIAA
CONTROL							
J2	15.560	0.895	0.762	0.193	3.050	14.275	26.248
J5	18.394	1.089	0.750	0.280	5.324	36.658	26.921
J8	16.590	0.814	0.651	0.197	2.230	16.303	17.067
J11	15.503	0.959	0.634	0.288	2.869	23.362	21.353
J13	10.271	0.792	0.655	0.338	3.017	20.438	27.575
J14	12.583	0.792	0.673	0.318	5.074	25.570	36.281
J15	12.450	0.776	0.866	0.241	3.326	18.393	21.390
J16	15.965	1.042	1.010	0.258	3.674	21.225	23.626
MEAN	14.676	0.895	0.750	0.264	3.571	22.028	25.058
+/-SEM	0.940	0.043	0.046	0.019	0.384	2.455	2.028
BMW							
J1	14.990	0.249	0.450	0.268	3.912	11.382	19.479
J3	23.744	0.445	0.836	0.320	3.019	23.859	24.374
J5	14.433	0.617	0.448	0.270	1.695	17.380	19.975
J6	11.526	0.192	0.392	0.213	2.289	14.671	17.248
J7	13.902	0.340	0.557	0.213	2.281	16.196	19.238
J9	9.623	0.599	0.328	0.271	2.268	15.687	16.991
J10	8.572	0.374	0.409	0.284	2.416	11.011	19.708
J12	10.473	0.364	0.343	0.193	2.439	13.584	19.744
MEAN	13.408	0.398***	0.470**	0.254	2.540*	15.471*	19.595*
+/-SEM	1.692	0.054	0.058	0.015	0.234	1.435	0.795

Values are ng/mg protein
* p<.05, ** p<.01, *** p<.001

Table 17. EFFECT OF BRAIN MISSILE WOUNDING (BMW) ON
BIOGENIC AMINES AND METABOLITES IN THE
ANTERIOR HYPOTHALAMUS (AH)

	NE	EPI	DA	DOPAC	HVA	5-HT	5-HIAA
CONTROLS							
J2	41.490	2.323	4.922	1.389	11.672	28.796	15.697
J5	35.256	2.300	3.955	1.032	8.351	19.762	11.122
J8	54.370	3.290	3.532	1.042	7.004	17.901	10.486
J11	41.558	3.620	7.498	2.197	6.811	24.163	10.735
J13	35.605	3.195	4.405	1.071	8.831	27.952	17.064
J14	40.172	3.327	4.726	1.501	12.116	23.889	14.198
J15	45.442	4.093	9.702	1.643	9.793	24.866	14.509
J16	42.269	3.385	5.562	1.778	13.596	27.858	15.229
MEAN	42.020	3.191	5.538	1.457	9.772	24.398	13.630
+/-SEM	2.134	0.216	0.732	0.146	0.877	1.390	0.889
BMW							
J1	46.591	1.834	5.799	1.875	8.956	21.589	11.751
J3	41.033	1.989	3.292	1.225	8.986	19.580	12.017
J5	34.062	1.947	3.547	1.074	4.429	23.520	12.088
J6	35.851	2.061	3.178	0.852	9.510	19.557	12.128
J7	43.338	2.397	5.335	1.485	9.741	20.075	11.808
J9	39.033	3.131	3.873	0.948	5.807	23.146	10.902
J10	34.883	2.015	3.819	1.132	5.154	22.232	10.412
J12	37.218	2.218	4.322	1.120	6.773	20.934	13.605
MEAN	39.001	2.199***	4.416	1.214	7.420	21.329	11.839
+/-SEM	1.553	0.147	0.338	0.116	0.752	0.550	0.333

Values are ng/mg protein
*** p<.001

Table 18. EFFECT OF BRAIN MISSILE WOUNDING (BMW) ON
BIOGENIC AMINES AND METABOLITES IN THE
POSTERIOR HYPOTHALAMUS (PH)

	NE	EPI	DA	DOPAC	HVA	5-HT	5-HIAA
CONTROLS							
J2	38.836	1.464	1.875	1.105	6.875	17.750	8.567
J5	45.064	1.672	3.950	2.506	2.859	23.550	17.035
J8	65.071	3.272	2.041	1.120	4.397	16.193	7.788
J11	52.801	2.491	9.311	2.692	8.971	21.482	12.457
J13	39.557	3.073	2.800	1.083	5.797	22.172	14.632
J14	37.141	3.097	9.716	3.797	8.303	17.821	12.862
J15	32.397	2.155	9.598	2.773	8.478	20.800	13.627
J16	45.684	2.144	10.080	1.882	8.467	19.935	14.042
MEAN	44.569	2.421	6.172	2.120	6.768	19.963	12.626
+/-SEM	3.622	0.240	1.345	0.350	0.789	0.891	1.089
BMW							
J1	29.342	0.779	2.229	6.518	5.652	17.452	7.561
J3	33.827	0.880	1.924	1.195	6.217	21.673	11.210
J4	20.394	0.668	1.848	0.949	3.630	18.775	11.343
J6	36.944	0.887	1.757	0.952	5.700	19.490	9.797
J7	29.850	1.033	2.844	1.315	5.721	16.031	8.472
J9	19.727	1.217	8.378	3.325	4.884	14.273	8.444
J10	11.993	0.379	2.577	1.470	4.403	15.416	9.451
J12	32.707	1.262	2.102	1.069	4.530	15.548	14.107
MEAN	26.848**	0.888***	2.957	2.099	5.092	17.332	10.048
+/-SEM	3.027	0.103	0.785	0.689	0.308	0.881	0.746

Values are ng/mg protein
** p<.01, *** p<.001

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THE EFFECT OF RAISED INTRACRANIAL PRESSURE ON
MECHANICAL CEREBRAL BLOOD FLOW REGULATION

INTRODUCTION

When normal cerebral blood flow (CBF) regulation mechanisms are present the brain is able to maintain a relatively constant flow despite a mean arterial blood pressure (MABP) reduction to about 60 mmHg by bleeding. Below this MABP level CBF regulation is impaired because cerebral vessels can not dilate further. At this point cerebral blood flow is MABP dependent. This response of CBF to graded hemorrhagic hypotension has been the classic test of CBF autoregulation and defines "mechanical" CBF regulation.(4,6,9)

Cerebral blood flow regulation also may be imparied when intracranial pressure (ICP) is elevated to greater than 70 mmHg(4,6,9,12).

In so far as cerebral circulation is concerned, cerebral perfusion pressure (CPP) is critical. CPP = MABP - ICP. If CPP is reduced too far CBF will fall. Cats with intact blood flow regulation mechanisms have been shown to autoregulate CBF with CPPs as low as 30 mmHg (12, Torbati, 1988 annual report).

In a prior work (DAMD17-86-C-6098, 27 April 1989) we evaluated mechanical CBF regulation in cats following a brain missile wound and observed that about half of so wounded cats could not maintain CBF when MABP was decreased by hemorrhagic hypotension, ie their mechanical CBF regulation was impaired. In all cases wherein brain wounded animals had been made hypotensive by bleeding to test mechanical CBF regulation, reinfusion of shed blood failed to improve CBF and, in fact, often decreased it.

In these prior experiments concerning the mechanical regulation of CBF after a brain missile wound we concluded that mechanical brain blood flow regulatory mechanisms were severely impaired but we were unable to ascertain how much of this impairment resulted from increased intracranial pressure which invariably accompanies a missile wound to the brain (1,2) and how much of the autoregulatory loss was from brain wound mechanisms per se.

The following experiments were designed to delineate the effect of increased intracranial pressure alone on mechanical CBF regulation. In the experiments herein being reported increased ICP was induced by an infusion of mock CSF into the cisterna magna. After ICP had been increased, mechanical CBF regulation was evaluated by measuring CBF during 3 levels of systemic hypotension induced by bleeding. As in brain wounded cats, animals in these experiments had CPPs lowered not only by ICP elevations but also by MABP reductions. In the present experiments, however, the physical effects of missile wounding were absent.

METHOD

SURGICAL PROCEDURE: The surgical and experimental procedures utilized were described in our previous reports as was our microsphere technique (See Final Reports: DAMD17-83-C-3145, 31 Dec 1985; DAMD17-86-C-6098, 21

Sep 1987). In the present experiments 8 mongrel cats of either sex (2.9 to 4.1 kg) were initially anesthetized i.p. with 30 to 40 mg/kg pentobarbital. After endotracheal intubation, one femoral artery was cannulated (PE 90 or 160) for MABP recording, periodic blood sampling, for bleeding to induce hypotension, and for blood reinfusion. The second femoral artery was used for placement of an intracradiac (PE 90) pigtail catheter for microsphere injection. Catheter tip placement within the left ventricle was determined by pulse pressure recordings and post-mortem examination. Both brachial arteries were cannulated (PE 50) for reference blood sampling and for administration of additional pentobarbital as needed during surgery and for infusion of gallamine (30 to 40 mg/cat) for paralysis. Each cat was then placed in a stereotaxic head apparatus and a fiber optic ICP probe (Camino, model 420) was inserted in the left parietal brain. Three EEG electrodes (miniature screws) were also implanted over the right and left parietal cortices. A 20 guage spinal needle was inserted into the cisterna magna and mounted on a stereotaxic arm. This needle was connected to a reservior containing mock CSF*.

TECHNIQUE FOR INCREASING ICP AND REDUCING MABP:

The container of mock CSF could be elevated above the cat's head in order to increase the cat's ICP up to a maximum of 120 mmHg. Graded hemorrhagic hypotension was achieved by arterial bleeding into pre-heparinized 20 ml disposable syringes. Blood in these syringes were kept at 37°C until used for blood reinfusion. In these experiments with unwounded cats, we attempted to simulate as closely as possible the increases in ICP which occured over time in cats which received a brain wound. After BMW usually there is an abrupt increase in ICP to very high levels (~ 150 mmHg) occuring within 2 to 3 min of wounding. The ICP in most cases then declines to 60 to 80 mmHg within 5 min and then continues to drop, stabilizing at 40 to 60 mmHg 20 to 40 mins after wounding. (See prior cited reports and 1988 Annual Report DAMD17-86-C-6098, April 27, 1989). Accordingly, in the present experiments ICP was raised by mock CSF infusion initially to greater than 100 mmHg but by five minutes after this initial rise ICP was reduced to ~82 mmHg by lowering the container containing the mock CSF. By 20 minutes mean ICP was decreased to 59 mmHg and by 40 minutes ICP was set at 42 mmHg. All during the time the ICP was artificially elevated by the mock CSF infusion MABP was gradually lowered in 3 stages from a control of 131 mmHg down to 70 mmHg. During blood reinfusion at 90 minutes ICP was again increased to 65 mmHg. At each of the 3 reduced blood pressure levels wherein mechanical CBF regulation was tested in the face of incresed ICP, MABP remained in the known autoregulatory range. We tried to keep CPPs relatively constant and at the lower limit of CPP which is consistant with CBF autoregulation, about 30 mmHg (12, Torbati, et al 1988 annual report).

* Chemical Composition: KCl 2.9 mEq; $MgCl_2$ 1.4 mEq; $CaCl_2$ mEq; NaCl 132 mEq; $NaHCO_3$ 24.6 mEq, Urea 6.7 mEq; Glucose 3.7 mEq

CBF MEASUREMENTS: In these experiments, total CBF and 14 rCBFs and cervical spinal cord blood flows were measured 5 times by means of: [153] Gd, [113] Sn, [103] Ru, [95] Nb and [46] Sc.

CBF #1 was measured after completion of all surgical procedures when intracranial and arterial blood pressures were normal. This control CBF measurement took place about 10 min before the induction of simultaneous intracranial hypertension and hemorrhagic hypotension.

CBF #2 was measured 5 minutes after induction of severe intracranial hypertension which consisted of an abrupt increase of ICP to 100-120 mmHg within 3 min followed by a gradual drop to an average ICP of 82 ± 5 mmHg at five minutes. At the same time, MABP was decreased by bleeding from a control of 131 to 111 mmHg. CPP was 29.7 mmHg. The mean volume of blood removed at this stage was 31 ± 3 ml/ cat or 8.9 ml/Kg body weight.

CBF #3 was measured 20 min after initiation of bleeding and intracranial hypertension. At this time ICP was reduced to 59 ± 2 mmHg while MABP was decreased to 96 mmHg. CPP was 35+3 mmHg. The mean volume of blood removed was 55 ± 5 ml/cat, about 16 ml/Kg.

CBF #4 was measured at 45 min after initiation of intracranial hypertension and bleeding. ICP was set at 42+1 mmHg while MABP was reduced to 70 mmHg (CPP=28+4 mmHg). The mean volume of blood removed at this final stage of hypotension was 87 ± 10 ml/cat equivalent to 25 ml/Kg body weight.

CBF #5 was measured after blood reinfusion in presence of a maintained increase in ICP. In our prior experiments (DAMD17-86-C-6098, 27 April 1989) testing mechanical CBF regulation following missile wounding, CBF autoregulation was impaired. Reperfusion of shed blood with restoration of MABP caused a parallel increase in ICP. This phenomenon has also been described in other instances where CBF regulation is impaired (Miller et al, 1973). Hence, in our present experiments we increased ICP gradually from 42+1 mmHg to 65+1 mmHg as blood was being reinfused and MABP was being raised from its lowest level of 70 mmHg to 148 mmHg. CBF #5 was, thus, measured following 90 min of maintained but variable levels of intracranial hypertension. Reinfusion and CBF measurements were successfully performed in 5 cats. Three cats were excluded because of instability of arterial PO_2 following of reinfusion (2) and accidental arterial bleeding in another.

These manipulations of ICP and MABP are summarized in Table 1 and diagramed in Figure 1.

STATISTICAL ANALYSIS: All data are presented as means $\pm$ SE. The results were first evaluated by analysis of variance. Comparison between control and subsequent CBFs was made by Tukey's test for repeated measurements. Selective comparisons between data in the present study and previous results in cats subjects to both brain missile wounding and hemorrhagic hypotension to test mechanical CBF regulation after brain wounding (DAMD17-86-C-6098, April 27, 1989) were made with unpaired t-test. Values of $p < 0.05$ were considered significant.

TABLE 1: MABP and ICP changes induced to test mechanical
CBF regulation in unwounded cats

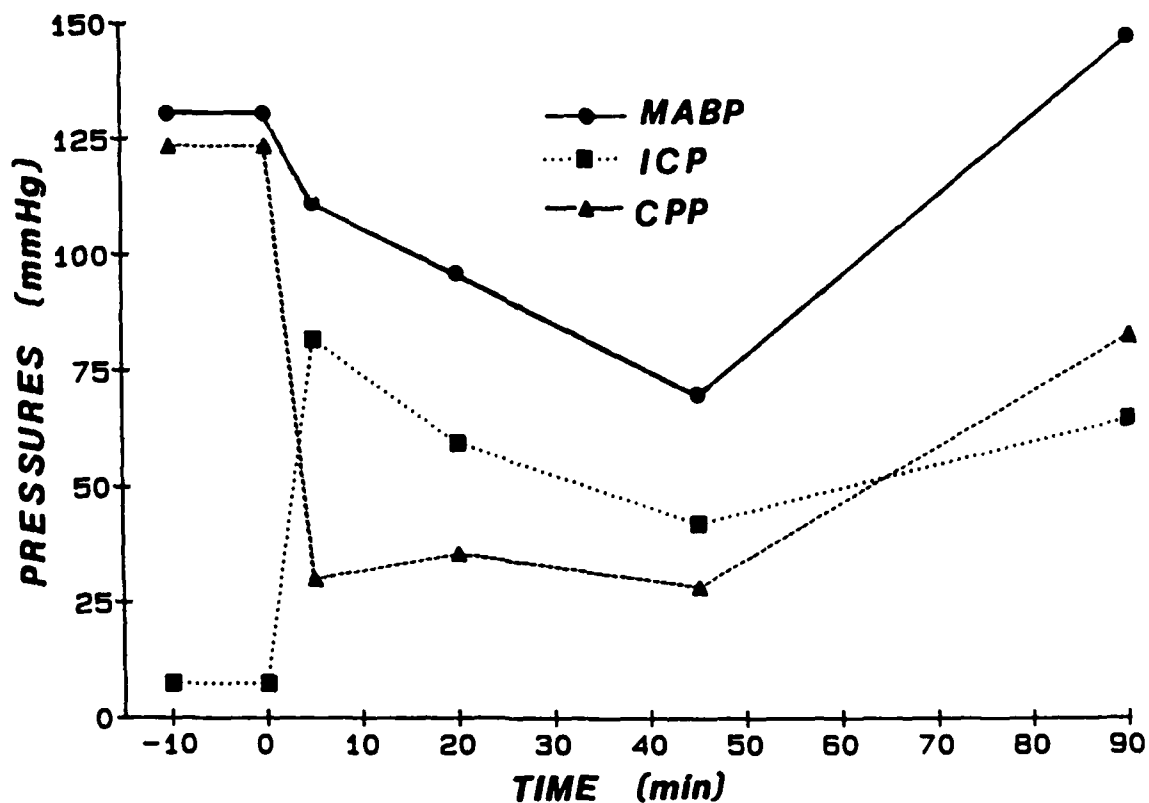| MANEUVER	CBF No.	MABP(mmHg)	ICP(mmHg)	CPP(mmHg)
None (Control)	1	130	7	123
raise ICP, lower MABP	2	111	82	29
lower ICP, lower MABP	3	96	59	35
lower ICP, lower MABP	4	70	42	28
raise ICP, raise MABP	5	148	65	83

Figure 1: Changes in ICP, MABP and CPP induced by artificially increasing ICP
and gradually reducing MABP by arterial bleeding in anesthetized ventilated
cats. These pressure changes simulate intracranial and systemic pressure
changes observed in brain wounded cats subjected to hemorrhagic hypotension.
n=8 up to 45 min and 5 at 90 min.

RESULTS

The control (Table 2), normotensive values of various physiologic variables in these 8 cats were all in the accepted range for pentobarbital anesthetized cats. With the onset of ICP elevation and bleeding these ventilated cats maintained a normoxic arterial PO_2 (95-107 mmHg). Their arterial PCO_2s and pHs, however, fell significantly during the course of bleeding; pH from a control of 7.35 to 7.18 and P_aCO_2 from 28 to 24 mmHg. This arterial acidosis persisted following completion of blood reinfusion pH was 7.20 (Table 2). The hematocrit was significantly reduced from 31 to 22% when MABP was reduced to 70 mmHg but returned to normal, 32%, following blood reinfusion.

Table 2: Changes in physiological variables in anesthetized ventilated cats during hemorrhagic hypotension associated with intracranial hypertension (5-45 min) and after blood reinfusion in presence of a maintained intracranial hypertension (90 min). Mean $\pm$ SE; n=8 for control and 5,20 and 45 min measurements; n=5 for reinfusion. *Significant, $p < 0.05$.

VARIABLES	CONTROL	5 MIN	20 MIN	45 MIN	90 MIN
pH	7.35	7.33	7.30	7.18*	7.20*
	0.01	0.01	0.01	0.04	0.02
[H+] (n/mol)	44.75	46.32	50.10	68.96*	63.46*
	1.44	1.00	1.52	7.62	3.52
PaO2 (mmHg)	106.63	106.00	106.13	106.88	95.20
	3.35	4.49	5.10	6.28	2.75
PaCO2 (mmHg)	28.49	26.63	26.57	23.61*	31.54
	1.56	1.78	0.58	1.34	1.34
MABP (mmHg)	130.50	111.25*	95.88*	70.00*	147.60*
	5.71	8.03	3.14	4.19	7.79
ICP (mmHg)	7.37	81.50*	59.37*	42.00*	65.00*
	0.60	5.30	2.31	1.35	0.84
CPP (mmHg	123.13	29.75*	35.25*	28.00*	82.60*
	5.61	6.36	3.05	3.60	8.03
CVR (CPP/CBF)	3.33	1.14*	1.22*	0.80*	2.44
	0.28	0.27	0.13	0.15	0.42
HEMATOCRIT (%)	30.75	31.00	27.75	22.13*	32.40
	2.12	1.85	2.45	1.86	3.53

Changes in Total CBF and CVR with Simultaneous Increased ICP and
Reduced MABP in Unwounded Cats: Tables 2 and 3 presents the salient data
from these set of experiments. When ICP was abruptly raised to > 100
mmHg and then decreased to 82 mmHg   total CBF fell significantly from a
control of 38.4 + 3 to 26.7 + 2 ml/100g/min. MABP, though reduced by
hemorrhagic hypotension from a control of 130 mmHg to 111 mmHg, was still
very adequate during this CBF measurement. CPP was about 30 mmHg, close
to the lowest level allowing CBF autoregulation. Though CVR showed an
appropriate decrease from 3.3 to 2.4 (autoregulatory mechanisms intact)
CBF still showed a reduction because of the excessively high ICP. The
next two CBF measurements were made 20 and 45 mins after the start of the
experimental period when MABP was successively lowered to 96 mmHg and
then to 70 mmHg in the face of ICPs ranging from 59 to 42 mmHg. Despite
the increased ICP, CBF showed no further significant reduction when MABP
was lowered and, in fact, returned very much to normal even though MABP
was decreased. This reflects the appropriate CVR decrease. Following
blood reinfusion and elevation of MABP above control, CVR increased
preventing hyperfusion of the brain.

Individual mean CBF vs. CPP relationships for all cats measured
during the control period, after 3 hemorrhagic hypotensive intervals, and
following reinfusion are illustrated in Figure 2. In no instance did
total CBF fall below 20 ml/100g/min which is above the ischemic level for
cats, -15 ml/100g/min (7,11).

Changes in rCBFs: Reflecting the total CBF change, significant rCBFs
reductions occurred in all investigated brain structures when ICP was 82
mmHg, (Table 3). Again, however, the residual regional flows were all
above ischemic levels and tended to recover and return to normal when ICP
was decreased to 59 mmHg and then 42 mmHg and corresponding MABPs were 96
and 70 mmHg.

Effect of blood reinfusion in presence of a moderate intracranial
hypertension:

Blood reinfusion produced a mild but significant systemic
hypertension (MABP 148 mmHg). The mean concomitant intracranial
hypertension of 65 mmHg did not prevent the necessary CVR rise from
accompanying the MABP increase which was required to keep CBF constant.

Table 3: Changes in rCBF and total CBF in anesthetized ventilated cats during hemorrhagic hypotension (5,20 and 45 min) and after blood reinfusion (90 min) in presence of maintained intracranial hypertension. Mean $\pm$ SE; n=8 reinfusion. *Significant, p<0.05.

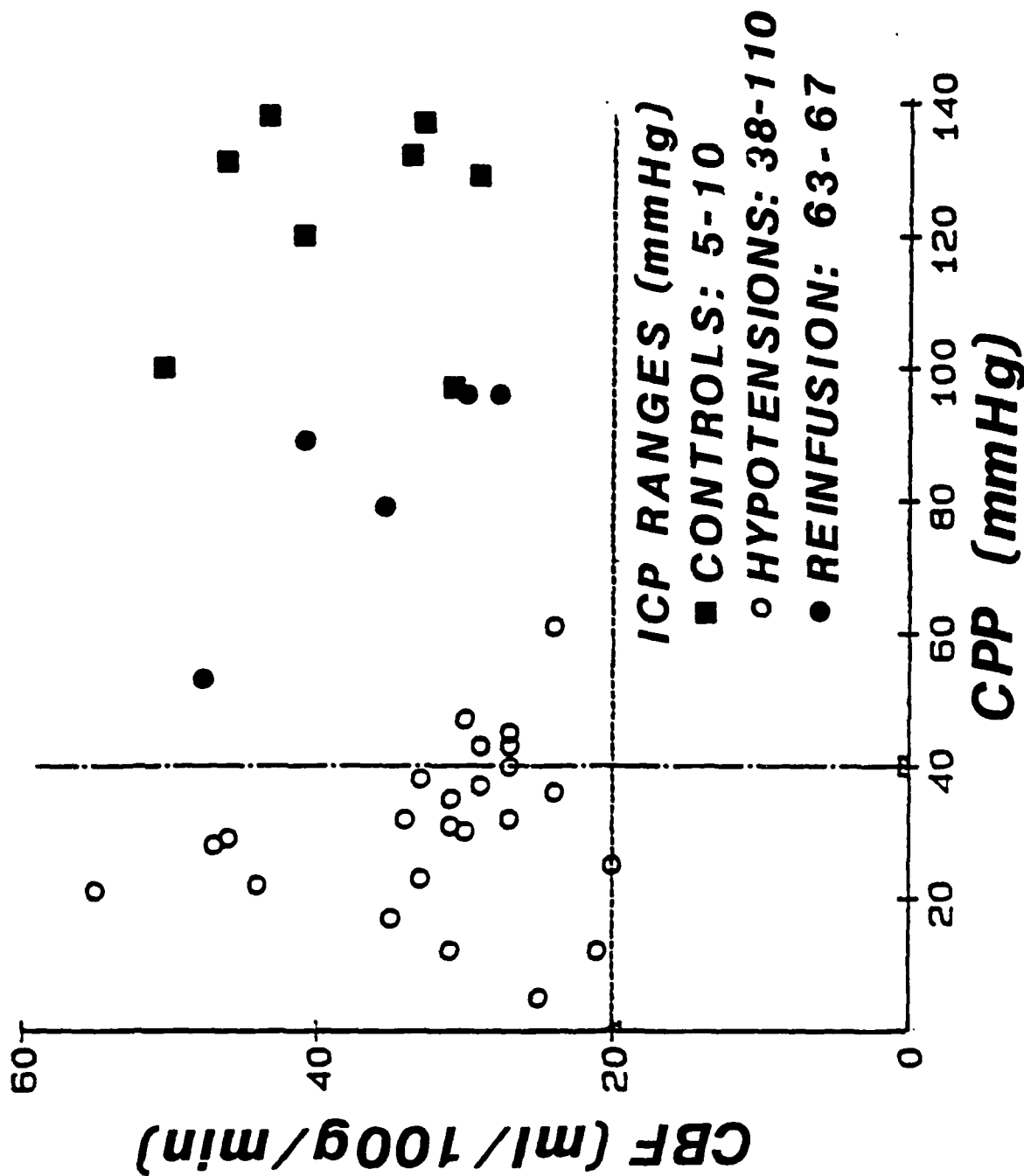| VARIABLES	CONTROL	5 MIN	20 MIN	45 MIN	90 MIN
MABP	130.5	111.3	95.9	70.0	147.6
ICP	7.4	81.5	59.4	42.0	65.0
CPP	123.1	29.8	36.5	28.0	82.6
FRONTAL CORTEX	43.50	30.69*	34.21	44.69	37.42
	3.55	2.37	3.25	4.31	3.98
PARIETAL CORTEX	42.52	26.41*	30.98*	39.34	34.24
	4.28	1.54	4.20	4.36	3.09
TEMPORAL CORTEX	29.24	26.56*	28.21	38.42	31.48
	2.52	1.52	2.15	3.26	4.01
OCCIPITAL CORTEX	47.56	26.57*	30.31*	39.44	42.34
	4.21	1.79	2.90	5.42	3.31
WHITE MATTER	34.66	23.10*	27.57	30.81	29.74
	3.19	2.15	2.93	2.00	2.55
CAUDATE NUCLEUS	55.36	42.17*	40.21*	58.01	52.00
	5.16	4.30	3.34	5.57	5.42
HIPPOCAMPUS	27.19	23.85*	27.70	36.55	27.18
	2.03	1.95	2.68	3.20	3.55
THALAMUS	45.24	35.24*	37.95	48.96	43.22
	4.78	2.91	2.77	3.57	3.27
RETICULAR	45.29	31.76*	33.10	43.95	41.02
FORMATION	4.21	2.99	2.84	3.45	4.11
BRAIN STEM	35.05	25.26*	28.26	35.60	32.50
& MEDULLA	2.08	1.67	2.49	3.71	4.18
CEREBELLUM GRAY	39.89	27.53*	32.37	36.67	46.42
	3.93	1.73	3.89	6.23	6.42
CEREBELLUM WHITE	42.34	27.13*	32.89	36.17	41.14
	2.95	1.48	3.70	5.69	7.30
TOTAL CBF	38.39	26.73*	30.27	37.87	36.25
	2.76	1.54	2.37	3.58	3.63

Figure 2: CBF-CPP relationship in individual cats during increased intracranial hypertension associated with 3 levels of systemic hypotension and after blood reinfusion. CBF remained above 20 ml/100g/min even when CPPs were below 30 mmHg indicating intact autoregulation.

DISCUSSION

The question these present experiments attempt to answer is whether the failure of mechanical CBF regulation following brain wounding occurs as a consequence of the missile wounding process per se or occurs mainly as a result of the brain-wound induced acute and chronic ICP elevations. To help resolve this issue the current experiments were undertaken wherein mechanical regulation of CBF was examined under conditions of elevated ICP alone. Table 4 indicates that under conditions of increased ICP alone mechanical CBF regulation remains intact.

In Table 5 data concerning the maintenance of CBF from our present experiments (Group C) are compared to that from other groups of cats also exposed to experiments which tested mechanical CBF regulation: Group A consists of normal unwounded cats exposed to hemorrhagic hypotension. ICP in these animals was normal. Group B_1 is comprised of cats which sustained a brain wound. Mean ICPs in these cats were never greater than 41 mmHg and cats in this group autoregulated their CBF when MABP was reduced by graded hemorrhagic hypotension. These cats could not, however, control ICP when shed blood was reinfused. Group B_2 consists of brain wounded cats in which mechanical CBF regulation was totally impaired; these cats could neither control CBF with induced hypotension nor ICP after shed blood reinfusion. ICP was elevated to 73 mmHg 5 minutes after wounding in this group. Groups A, B_1 and B_2 came from our prior work on mechanical regulation of CBF following brain wounding presented in our 1988 yearly report, DAMD17-C-60C ₅, 27 April 1988.

The basic results of these experiments are briefly recapitulated in Table 4.

TABLE 4: Mechanical CBF regulation when ICP is elevated
(derived from tables 2 and 3)

Time (min)	MABP	ICP (mmHg)	CPP	CBF (ml/100g/min)	CVR (CPP/CBF)
-10	130	7	123	38	3.3
+5	111	82	29	27*	1.1*
+20	96	59	35	30	1.2*
+45	70	42	28	38	0.8*
+90	148	65	83	36	2.4

* Significant (p<0.05) change from control

These results indicate that unwounded cats cannot completely maintain CBF with ICPs > 80 mmHg even though MABP, is adequate. Under these circumstances even though CVR shows an appropriate decrease, CBF tends to fall, presumably because the effect of the greatly elevated ICP predominates. Mechanical autoregulation remains intact, however, even though ICPs are elevated between 42 and 59 mmHg because when MABP is decreased to 70 mmHg, CBF remains normal via CVR reduction mechanisms which remain operative even though CPPs are at the lower levels compatable with intact CBF regulation (28-35 mmHg).

TABLE 5: Mechanical CBF regulation in normal cats, brain wounded cats and unwounded cats with increased intracranial pressure

Group	Physiologic Conditions	Time (min)	MABP (mmHg)	ICP (mmHg)	CPP (mmHg)	CBF (ml 100g min)	CVR
		control	116	6	110	39	3.6
	Unwounded+	+5	89	5	84	34	2.9
A	Hemorrhagic	+20	68	5	63	35	2.1
	Hypotension	+45	48	4	44	31	1.6*
	N=8	+90	122	8	114	43	3.2
		control	115	5	110	37	3.2
	Wounded+	+5	98	41	57	35	1.7
B_1	Hemorrhagic	+20	62	26	36	34	1.2
	Hypotension	+45	38	16	22	31	0.7*
	N=4	+90	82	57	25	22*	1.1*
		control	135	8	127	42	3.0
	Wounded+	+5	111	73	38	14*	2.7
B_2	Hemorrhagic	+20	64	50	14	11*	1.3
	Hypotension	+45	47	41	6	7*	0.8
	N=6	+90	91	62	29	4*	7.2
	Unwounded	control	131	7	124	38	3.3
C	ICP +	+5	111	82	29	27*	1.1*
	Hemorrhagic	+20	96	59	37	30	1.2*
	Hypotension	+45	70	42	28	38	0.8*
	(N= 5-8)	+90	148	65	83	36	2.4

* Significant (p<0.05) change from control

Unwounded cats (Group A) subject to hemorrhagic hypotension were able to maintain CBF by decreasing CVR. The B_1 group of brain wounded cats was also able to maintain CBF when MABP was lowered by bleeding. Mean ICP in this group was -40 mmHg 5 minutes after wounding. CVR appropriately decreased and remaind so throughout the hypotensive period. With reinfusion of blood (+90 min), however, a large part of the MABP increase was transmitted intracranially and ICP showed more than a 3 folds rise. Despite reinfusion and restoration of MABP, therefore, CBF fell even though CPP remained in or just below the autoregulatory range. This rise in ICP with blood reinfusion indicates a partial loss of autoregulatory ability. The B_2 group of brain wounded cats with a much higher mean initial ICP of 73 mmHg showed an abrupt fall off of CBF as soon as MABP was lowered even slightly from control levels to 111 mmHg. CVR, though reduced somewhat, initially was not reduced enough to maintain CBF which fell to 14 ml/100g/min despite MABP and CPPs which should have allowed mechanical CBF regulation. Mechanical blood flow regulation clearly failed in this group. With blood reinfusion ICP rose dramatically because of transmission of MABP into the intracranial compartment. This indicates failure of another aspect of autoregulation. Though MABP was restored CBF did not rise owing to completely deranged blood flow autoregulation mechanisms. Unwounded cats with ICP elevation up to 59 mmHg in the present experiments, Group C, were able to maintain CBF with MABP reductions to 70 mmHg.

The effect of brain wounding with concomitantly elevated ICP on CBF as opposed to elevated ICP alone on CBF is best seen by comparing groups B_2 and C 5 minutes after wounding, table 6 and Figure 3 comparing the percentage change in rCBFs between wounded and unwounded cats also provides insight into the ability of unwounded cats to autoregulate CBF despite elevated ICP.

Table 6: Comparison of mechanical CBF regulation between brain wounded cats with increased ICP and unwounded cats with increased ICP

	Group B_2 Brain Wound + ICP			Group C Normal + ICP		
	Control	5 min Post Wound	Reinfusion	Control	5 min Post Wound	Reinfusion
MABP mmHg	135	111	91	131	111	148
ICP mmHg	8	73	62	7	82	65
CPP mmHg	127	38	29	124	29	83
CBF ml/ 100g/min	42	14*	4*	38	27*	36
CVR	3.7	2.7	7.2	3.3	1.1*	2.4

* Significant (p <0.05) change from control

Animals in group B$_2$ which sustained a brain wound with concomitant elevation in mean ICP to 73 mmHg could not effect a substantial CVR reduction after MABP reduction and consequently CBF fell precipitously into the ischemic range. Cats in group C maintained CBF well above ischemic levels a despite a markedly elevated ICP up to 82 mmHg. CVR was appropriately reduced from a pre-wound level of 3.3 to post wound level 1.1. These data indicate that brain wounding impairs the mechanical autoregulatory response to a far greater degree than does just an ICP rise by itself and suggests that the impairment of mechanical CBF regulation consequent to missile wounding cannot be explained solely on the basis of increased ICP.

Figure 3 compares the percent change in rCBFs between wounded and unwounded cats subjected to hemorrhagic hypotension and approximately the same level of increased ICP, 59 to 60 mmHg. These data are derived from pooled group B$_1$ and B$_2$. Significant reductions occured in many rCBFs in wounded cats but only 3 cortical areas in unwounded cats exhibited a decrease.

Another component of successful CBF autoregulation is the exclusion of a large component of the MABP from the intracranial compartment: normally MABP averages ~100 mmHg while ICP is only 7-15 mmHg. Neither group of brain wounded cats (B$_1$ or B$_2$) could exclude MABP from the intracranial compartment with blood reinfusion while cats in group C could despite a persistantly elevated Mean ICP, (Tables 5 and 6)

The successful exclusion of MABP from the intracranial space with blood reinfusion and the normal CVR response exemplifies another aspect of mechanical CBF regulation not disturbed at all by ICP elevations alone but clearly deranged by brain wounding plus increased ICP. This further suggests that increased ICP by itself does not underline the mechanical CBF disruption following brain wounding.
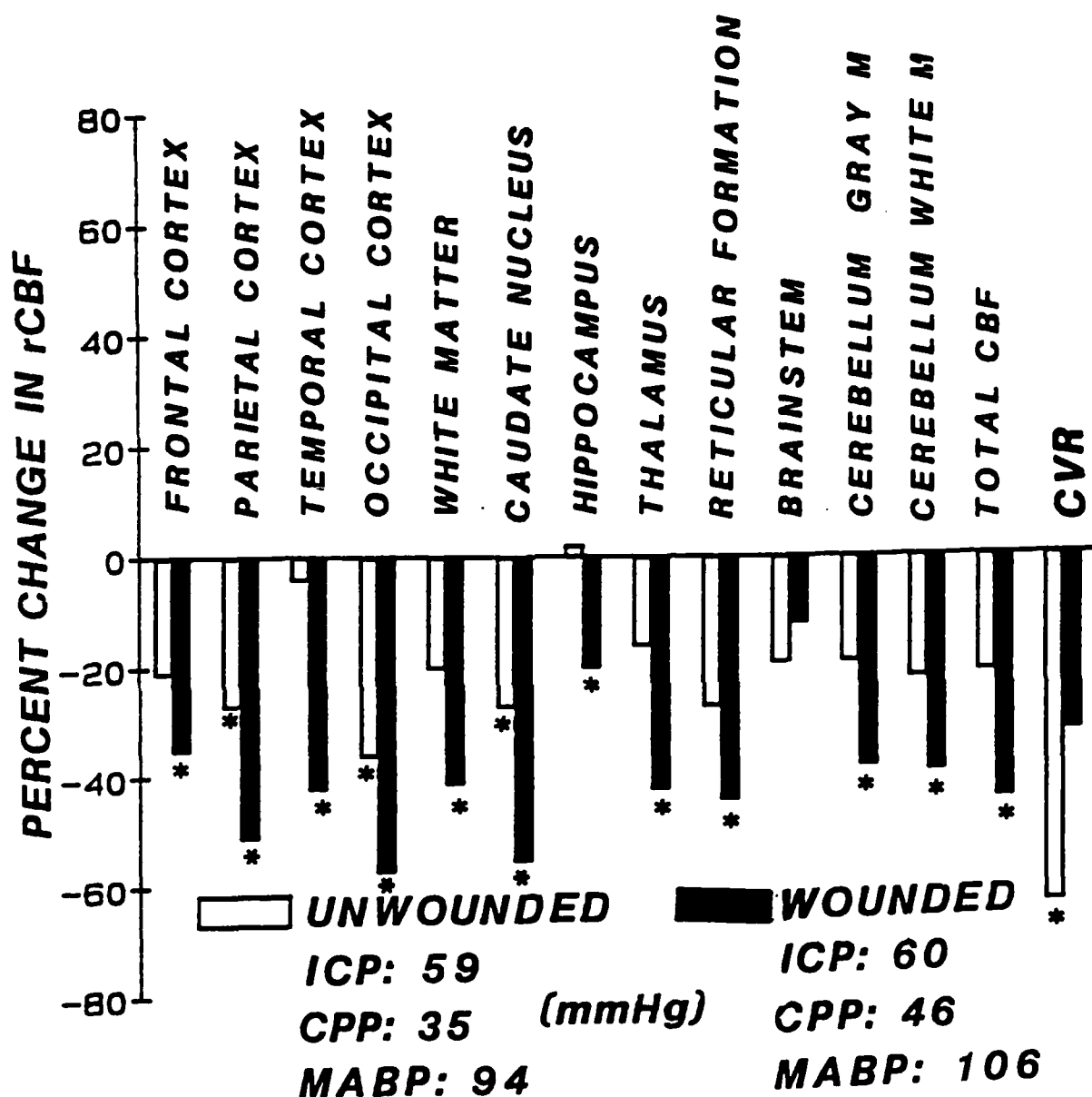
Figure 3: Percent changes in rCBFs between wounded and unwounded cats subjected to hemorrhagic hypotension and approximately the same levels of increased ICP. Brain wounded cats with elevated ICP were not able to autoregulate rCBF. Data from wounded cats at 5 minutes; post wound data are compared with data from unwounded cats 20 minutes after ICP increase

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